# Mason, P. G., D. R. Gillespie & C. Vincent (Eds.) 2013. Proceedings of the Fourth International Symposium on Biological Control of Arthropods. Pucón, Chile, 4–8 March 2013, 381 p.


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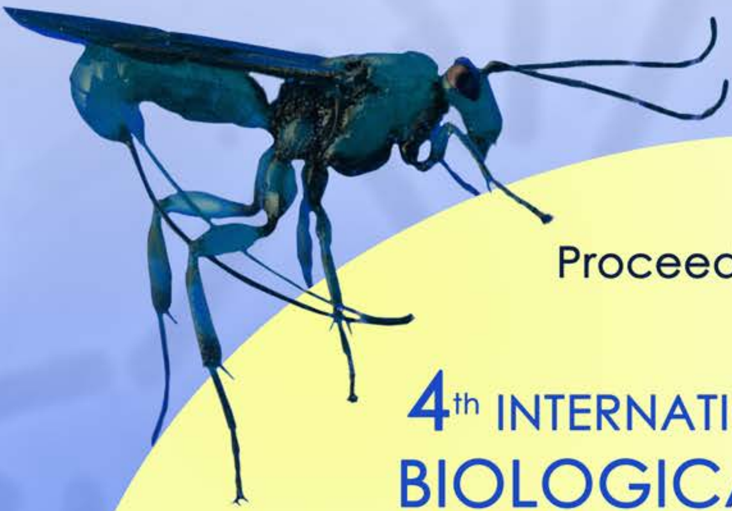

Proceedings of the

# 4th INTERNATIONAL SYMPOSIUM on BIOLOGICAL CONTROL OF ARTHROPODS

Pucón, Chile

March 4-8, 2013

Edited by
Peter G. Mason, David R. Gillespie & Charles Vincent
Agriculture and Agri-Food Canada

IUFRO

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

**The correct citation of this work is:**
Peter G. Mason, David R. Gillespie and Charles Vincent (Eds.). 2013. Proceedings of the 4th International Symposium on Biological Control of Arthropods. Pucón, Chile, 4-8 March 2013, 380 p.

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# 4th INTERNATIONAL SYMPOSIUM ON BIOLOGICAL CONTROL OF ARTHROPODS

### Pucón, Chile, March 4-8, 2013

## PREFACE

The Fourth International Symposium on Biological Control of Arthropods, held in Pucón – Chile, continues the series of international symposia on the biological control of arthropods organized every four years. The first meeting was in Hawaii – USA during January 2002, followed by the Davos - Switzerland meeting during September 2005, and the Christchurch – New Zealand meeting during February 2009. The goal of these symposia is to create a forum where biological control researchers and practitioners can meet and exchange information, to promote discussions of up to date issues affecting biological control, particularly pertaining to the use of parasitoids and predators as biological control agents. This includes all approaches to biological control: conservation, augmentation, and importation of natural enemy species for the control of arthropod targets, as well as other transversal issues related to its implementation.

To this end, 19 sessions have been organized in order to address the most relevant and current topics in the field of biological control of arthropods, delivered by invited speakers, contributed talks and poster presentations. Some of these topics have remained as important issues since the first meeting, like risk assessment in classical biological control, non-prey feeding in conservation biological control and the compatibility of GMO's with biological control. But also, as new tools and environmental concerns arise, some fresh topics have emerged. Among them are the use of classical biological control for the protection of biodiversity, the integration of conservation biological control with wildlife conservation, the effect of climate change on biological control, the potential of enhancing augmentative releases of beneficial organisms with radiation and the use of volatiles to monitor or manage natural enemies.

Another important goal of these meetings has been to be truly international, and this is why every conference so far has been organized on a different continent. This year we are excited in having achieved this goal despite the many world crises, by having participants from over 30 countries and all continents. But we are particularly happy for the many works and participants from South America, a region that in the past has been poorly represented in these symposia. As a result, this meeting represents an opportunity for creating and expanding networks between researchers worldwide.

Thus we expect that the 4th International Symposium on Biological Control of Arthropods would be an important milestone to keep moving forward the research and practice on biological control of arthropods, and with this helping to improve the sustainability of managed systems as well as aiding in the protection of biodiversity on the planet.

**ACKNOWLEDGEMENTS**

We are very grateful to the following individuals for their help in organizing ISBCA IV:

**SCIENTIFIC SESSION ORGANIZING COMMITTEE MEMBERS**
**Session 1**: Peter Mason (Agriculture and Agri-Food, Canada), Dave R. Gillespie (Agriculture and Agri-Food, Canada) & Jian J. Duan (USDA-ARS, USA) (hosted by IOBC Global Working Group on Exotic Biological Control Agents). **Session 2**: Thibaut Malausa (INRA Sophia Antipolis, France) & Blas Lavandero (Universidad de Talca, Chile). **Session 3**: Carlos Wilcken (Universidade Estadual Paulista, Brazil) & Marc Kenis (CABI, Switzerland) (hosted by IUFRO Working Party 7.03.13 Biological control of forest insects and pathogens). **Session 4**: Roy Van Driesche (University of Massachusetts, USA) & Mark Hoddle (University of California – Riverside, USA). **Session 5**: Jorge

Hendrichs & Andrew Parker (Joint FAO/IAEA Division of Nuclear Techniques, Austria). **Session 6**: Eric Riddick (USDA-ARS, USA) & Vanda H. P. Bueno (Federal University of Lavras, Brazil). **Session 7**: Kevin Heinz (Texas A&M University, USA) & Michael Parella (Univesity of California – Davis, USA). **Session 8**: Marc Kenis (CABI, Switzerland) & Vincent D'Amico (USDA Forest Service, USA) (session is hosted by IUFRO Working Party 7.03.13, Biological control of forest insects and pathogens). **Session 9**: Marta Montserrat Larrosa (Spanish National Research Council, Spain); Josep Jacas Miret (Universitat Jaume I - Castelló, Spain) & Tim Haye (CABI, Switzerland). **Session 10**: Nick Mills (University of California – Berkeley, USA), Don Weber (USDA-ARS, USA) & Tania Zaviezo (Universidad Católica de Chile, Chile). **Session 11**: David G. James (Washington State University, USA) & Steve Wratten (Lincoln University, New Zealand). **Session 12**: Audrey Grez (University of Chile, Chile) & Tania Zaviezo (Universidad Católica de Chile, Chile). **Session 13**: Don Weber & Jon Lundgren (USDA-ARS, USA). **Session 14**: Arne Janssen & Maurice Sabelis (University of Amsterdam, The Netherlands). **Session 15**: Ada Szczepaniec (South Dakota State University, USA) & Jonathan Lundgren (USDA-ARS, USA). **Session 16**: Matthew H. Greenstone (USDA-ARS, USA) & Paula M. Shrewsbury (University of Maryland-College Park, USA). **Session 17**: Vanda H. P. Bueno (Federal University of Lavras, Brazil) & Jose Belda (Koppert, Spain). **Session 18**: Joerg Romeis (Agroscope Reckenholz, Switzerland) & Anthony M. Shelton (Cornell University, USA).

## SCIENTIFIC COMMITTEE

Members of the Scientific Committee are thanked for providing input into session themes and other aspects of the meeting. The Committee: Barbara Barratt (AgResearch Ltd., New Zealand), Jacques Brodeur (University of Montreal, Canada), Roy Van Driesche (University of Massachusetts, USA), Mark Hoddle (University of California – Riverside, USA), Ulrich Kuhlmann (CABI, Switzerland), Nick Mills (University of California – Berkeley, USA), Alberto Urbaneja (Instituto Valenciano de Investigaciones Agrarias, Spain), Tania Zaviezo (Universidad Católica, Chile)

## REGIONAL ORGANIZERS

We thank Regional Organizers for coordinating ISBCA IV advertising, soliciting interest in the conference, and for helping with local inquiries. Regional Coordinators included: Europe: Ulli Kuhlmann (CABI, Switzerland). USA: Mark Hoddle (University of California – Riverside, USA). Southern Africa: Rami Kfir (Agricultural Research Council, Pretoria, South Africa). New Zealand: Barbara Barratt (AgResearch Ltd., New Zealand). Asia: Feng Zhang (CABI, China). South America: Marcos Gerding (Biobichos, Chile).

## LOCAL ORGANIZATION and COMMITTEE

Ernesto Cisternas (Instituto Nacional de Investigaciones Agropecuarias), Rodrigo Chorbadjian (Universidad Católica de Chile), Audrey Grez (Universidad de Chile), Blas Lavandero (Universidad de Talca), Marcos Gerding (Biobichos Ltda.), Tania Zaviezo (Universidad Católica de Chile).

We are also grateful to the people that help us with informational recourses and accounts: Jorge Ponce Toloza (Facultad de Matemáticas-UC), Alexis Ortiz, Beatriz Placencio, Eva Muñoz y Alejandra Fuentes (Facultad de Agronomía e Ingeniería Forestal – UC), and to the proceedings editors Peter Mason, David Gillespie, Charles Vincent (Agriculture and Agri-Food Canada), for their diligent and thorough work. Pierre Lemoyne worked on the page layout and Julien Saguez on the cover design.

CONFERENCE LOGO Jorge Ponce Toloza

Starting 2013, all Proceedings of ISBCA will be available freely (as pdfs) on several servers.

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# Session 1: Risk Assessment in Arthropod Biological Control: Where are we?

## Risk assessment in arthropod biological control: Where are we?

Peter G. Mason[1], Dave R. Gillespie[2], and Jian J. Duan[3].

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Assessment of the risk of introducing arthropods as biological control agents (BCAs) is a relatively new area of research. This field has gained importance in response to criticisms that unintended impacts of BCAs were occurring more frequently than was being reported. Arguments promoting the need for better assessment of risk of introducing arthropod biological control agents continue to be put forward (e.g. Simberloff 2005, Holland et al. 2008). In response, regulatory agencies around the world have tightened their requirements for approval of releases of new BCAs (reviewed by Hunt et al. 2008, 2011). The research community working with arthropod BCAs has proposed and developed methods to produce the data required by regulatory agencies. The publications by Van Driesche and Reardon (2004), Bigler et al. (2006), and van Lenteren et al. (2006) are examples where these proposed methods have been published.

Few studies have been published that have utilized these new methodologies. In general, these new approaches require more time and resources to conduct and thus, most studies based on the methods are ongoing. Barratt et al. (2010) summarized progress in risk assessment of arthropod biological control agents and noted several key outcomes of research to that point: 1) that accurate identifications of BCAs are crucial, as is the characterization of biotypes, and the preservation of voucher specimens in perpetuity; 2) that “host range testing methods need to be tailored to the unique biology of each biological control agent”; 3) that a tiered approach should be taken in which initial tests maximize the chances of detecting any non-target effects and subsequent tests, if required, then determine the mechanisms for any observed effects; 4) that post-release monitoring is key to validating risk predictions based on laboratory and field studies in the native range of the biological control agent during the risk assessment phase; and 5) that population models can provide insight into the potential impacts of target and non-target species.

The presentations in this session address the proposed new methods for risk assessment. Todd et al. propose a decisión-support method for selecting non-target species for host range testing. Haye et al., and Duan examine how well methods for selection of non-target species for host range studies, and choice and no-choice tests work in practice. Gariepy examines how new methods, e.g. molecular tools, can be used to improve risk assessment. Haye et al. and Barratt et al. assess the importance of studies in the BCA’s native range for determining the potential host range of a candidate biological control agent. Bromfield and Wratten, and Duan present some new ideas for quantifying impacts and risk. Overall, it is our hope to shine some light into the black box approach for non-target testing and risk assessment that was described by van Lenteren et al. (2006). It is clear that much progress has been made in the science of risk assessment in biological control, and that this field is developing rapidly.

## References

Barratt, B. I. P., F. G. Howarth, T. M. Withers, J. M. Kean, and G. S. Ridley. 2010. Progress in risk assessment for classical biological control. Biol. Control 52: 245–254.

Bigler, F., D. Babendreier, and U. Kuhlmann (eds.) 2006. Biological Control of Arthropods Using Invertebrates: Methods for Environmental Risk Assessment. CABI Publishers, Wallingford, UK.

Holland, B. S., C. C. Christensen, K. A. Hayes, and R. H. Cowie. 2008. Biocontrol in Hawaii: A response to Messing (2007). P. Hawaiian Entomol. Soc. 40: 81–83.

Hunt, E.J., U. Kuhlmann, A. Sheppard, T-K. Qin, B. Barratt, L. Harrison, P.G. Mason, D. Parker, J. Goolsby, and B. Flanders. 2008. Regulation of invertebrate biological control agents in Australia, New Zealand, Canada and the USA: recommendations for Europe in its pursuit of a harmonised regulatory system. J. Appl. Entomol. 132: 89-123.

Hunt, E. J., A. J. M. Loomans, and U. Kuhlmann. 2011. An international comparison of invertebrate biological control agent regulation: What can Europe learn?, pp. 79–112 *In* R-U. Ehlers (ed.) Regulation of Biological Control Agents. Springer, Dordrecht, Netherlands.

Simberloff, D. 2005. The politics of assessing risk for biological invasions: The USA case study. T. Ecol. Evol. 20: 216–222.

Van Driesche, R. G. and R. Reardon (eds.). 2004. Assessing host ranges for parasitoids and predators used for classical biological control: A guide to best practice. FHTET-2004-03, United States Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team, Morgantown, West Virginia, USA.

van Lenteren, J.C., J. Bale, F. Bigler, H.M.T. Hokkanen, and A.J.M. Loomans. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. Annu. Rev. Entomol. 51: 609–634.

# Selection of non-target species for risk assessment of entomophagous biocontrol agents using an automated decision-support system

Jacqui H. Todd[1], Barbara I.P. Barratt[2], and Louise A. Malone[1]

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The release of entomophagous biological control agents (BCAs) into a new country can pose risks to the existing insects in the release area. Risk assessment of these BCAs can be difficult and costly and usually only a few of the at-risk non-target species can be selected to undergo biosafety testing. To provide some decision-support around this selection process in New Zealand, the PRONTI (priority ranking of non-target invertebrates) species selection method has been adapted for use with entomophagous BCA.

The PRONTI method was originally developed and used to aid the selection of non-target invertebrate species to undergo biosafety testing with transgenic plants (Todd et al. 2008, Barratt et al. 2010, Malone et al. 2010). In New Zealand, the legislative requirements for the assessment of risk from exotic BCAs is the same as that for transgenic plants, since both are considered to be “new organisms” under the Hazardous Substances and New Organisms (HSNO) Act (Anon 1996). This suggests that the selection criteria used by the PRONTI method to prioritise non-target invertebrate species for biosafety testing with transgenic plants might also be applied to entomophagous BCA. Five selection criteria are used by the method to prioritise species for risk assessment testing:

1) The potential hazard posed by the new organism to each non-target species
2) The estimated degree of exposure predicted for each non-target species
3) The potential ecological impact that may occur if the non-target species’ population was affected by the new organism
4) The anthropocentric value of each non-target species
5) The testability of each non-target species

The first two criteria are used to assess the risk posed by the new organism to each non-target invertebrate species and as such are the primary criteria driving the prioritisation of non-target species for biosafety testing. The third criterion assesses the possible environmental impact of an interaction between the new organism and the non-target species’ population and involves an assessment of the size of the non-target species’ food web, its biomass, and its ability to mitigate the risk posed by the new organism. The fourth criterion ensures valued invertebrate species (e.g., rare, iconic or native species, ecosystem service providers or income generators) are prioritised for testing, and the fifth criterion acknowledges that non-target species that are easy to collect and rear in captivity would be easier to test and could be prioritised, although it is important that this is not a major driver of non-target species selection (USEPA 1998). Each of these criteria has been used in the selection of non-target species to undergo biosafety testing with entomophagous BCA by both New Zealand and international researchers (Van Driesche and Hoddle 1997, Bourchier 2003, van Lenteren et al. 2003, Van Driesche and Reardon 2004, Kuhlmann et al. 2005, Kuhlmann et al. 2006, Berndt et al. 2009, Toepfer et al. 2009). This suggests that these criteria could also be used by the PRONTI method to prioritise species for biosafety testing with entomophagous BCA in New Zealand.

The basis of the PRONTI method is a Microsoft® Access database (called the Eco Invertebase) that has been developed to enable the collation of published information on invertebrate taxa found in New Zealand. The database currently contains biological, ecological and physiological information on more than 1400 of the country's invertebrate taxa, as well as information on the likely interactions between each taxon and a new organism. To enable the PRONTI method to be used with BCAs the Eco Invertebase was adapted so that it could collate all the information on the non-target species that would be needed by the method to prioritise the species for biosafety testing with a BCA (see Table 1 for a summary of the data collated in the Eco Invertebase). The primary changes to the database involved gathering information on the potential direct and indirect hazards posed by an entomophagous BCA (which are quite different from those posed by a transgenic plant), the possible routes of exposure, and the attributes an invertebrate might use to avoid exposure to the hazards posed by the BCA.

To enable the PRONTI method to use the information in the Eco Invertebase, scores were assigned to each piece of information based on how well it informed each selection criterion. For example, a species that was known to be easy to rear in captivity was given a high score for the fifth criterion (testability), and a rare native species received a high score for criterion four (anthropocentric value). The scores were assigned on a scale of 0 to 10, with a score of 10 assigned to species' attributes that were most informative to the criterion of interest. Since there was much variation in the information available on each species, there were many cases where one or more attributes were unknown. In all such cases, a score of 5 was applied, since the species' actual attribute may have been of higher or lower value to the measurement of the criterion. This allowed data gaps to be accounted for and little known species were still considered for prioritisation by the method.

The scores thus obtained by each species for each attribute in the database were then combined to produce a score for each criterion (please see Todd et al. (2008) for more details on how the scores were combined). The following equation was then applied to determine a PRONTI score for each species:

$$\text{PRONTI score} = \frac{(\text{Hazard} \times \text{Exposure})}{(\text{Species' Resilience})} \times (\text{Status} + \text{Value} + \text{Testability})$$

Where:

Hazard = the score obtained by the non-target species for all the attributes used to estimate the potential hazard posed by the BCA to that species;
Exposure = the score obtained by the non-target species for all the attributes used to estimate the amount of exposure to the BCA for that species;
Species' Resilience = the score for the species' attributes that could reduce the risk the BCA poses to the species (e.g., the species' ability to disperse away from the BCA, or a high intrinsic rate of population increase).
Status = the score for the non-target species' ecological status (i.e., its estimated biomass, ecological function, and number of food web links to other species in the ecosystem).
Value = the score for the non-target species' estimated value to humans;
Testability score = the score for the ease with which the non-target species could be studied in the laboratory.

Note that the hazard and exposure scores were multiplied together to produce a 'risk score' since if either score was zero (i.e. there was either no potential hazard or no likelihood of exposure) for a species, then the risk score for that species would be zero. The risk score was divided by the resilience score since each species' resilience mechanisms may reduce the hazard or the exposure level. The status, value, and testability scores were multiplied by the risk score as the estimated risk was the primary driver in the prioritisation of the non-target species (i.e. where there was no risk, the species would not need to undergo biosafety testing). The status, value and testability scores were additive, since a species that scored highly in all three should be prioritised for testing, while a zero score for any of these criteria would not necessarily indicate that the species was not a high priority for testing. The status, value, and testability scores in the equation could be weighted by adjusting the total scores obtained for each in relation to each other and to the other variables in the equation. For instance, if the value and testability criteria were considered equally important in the risk assessment, the total scores obtainable by a non-target species for these criteria would be made equal. To aid in the interpretation of the scores, the uncertainty with which the scores for each of the five criteria were calculated was determined for each species. Uncertainty was calculated as the percentage of unknown or estimated attributes that were used to quantify each selection criterion for each species.

Each non-target species' PRONTI score was then used to prioritise the species for biosafety testing with the BCA, with the species obtaining the highest PRONTI scores at the top of the list. The resulting prioritised list could be used to direct the selection of species to undergo biosafety testing with the BCA during the risk analysis phase of the risk assessment process. The results from these tests could then be entered into the Eco Invertebase to be available for further iterations of the model, thus improving the prioritised list of species as necessary. Consequently, the PRONTI method can be used to aid in the selection of species to undergo biosafety testing before the release of a BCA, thus reducing the likelihood of unexpected ecological impacts from the introduction of the BCA to the environment. A proof of concept study is currently in progress which will compare current methods of non-target species selection with the PRONTI output.

Table 1: Information held to the Eco Invertebase to enable the PRONTI (priority ranking of non-target invertebrates) method to prioritise invertebrate taxa for biosafety testing with entomophagous biological control agents

| **Information collated for each non-target species:** |
|---|
| Taxonomy including synonyms and common names |
| Feeding guild and niche |
| Known food species and natural enemies |
| New Zealand endemism, distribution and abundance |
| Worldwide distribution |
| Density in New Zealand ecosystems and dry weight |
| Mobility of all life stages |
| Reproductive rate and seasonality |
| Diseases, cryptic colouring and avoidance or defensive behaviours |
| Resource requirements, including the use, or construction of, shelters |
| Aesthetic, symbolic, economic, cultural or ecological value (to humans) |
| Ability to maintain, test, or rear in the laboratory and ease of collection from the field |
| **Information collected for each biological control agent:** |
| Agent’s taxonomy including biotype |
| Type of agent (e.g., predator, parasitoid) |
| Type of control required (e.g., augmentative, classical) |
| Use of the agent in other countries or regions and its effectiveness |
| Distribution, abundance and seasonality of the agent in its area of origin or other regions where it has been released |
| Mobility of the agent |
| List of species the agent is known to attack (i.e., both target and non-target) |
| Identification of any relationship/s between the species the agent is known to attack (e.g., all are in the same genus, all feed on the same plant species) |
| Expected distribution of the agent in New Zealand and supportive modelling data |
| Information on similar agents introduced to New Zealand and their non-target impacts |
| Identity of the target species, the life stages likely to be attacked, the dispersal potential of those life stages, the ecosystems in which they are found, and the distribution of the target’s known food species in the area of introduction |
| **Information on the interaction between the agent and each non-target species:** |
| Taxonomic and phylogenetic relatedness of the non-target species to the target species |
| Other relationships between the non-target and target species (e.g. feeding niche, food preferences, predation) |
| Taxonomic relatedness of the non-target species to other species known to be attacked by the agent |
| Taxonomic relatedness of the non-target species to the agent (i.e. risk of hybridisation) |
| Results of previous host specificity tests conducted between the agent and the non-target species or likelihood of the non-target species being attacked by the agent given what is known about the agent |
| Likelihood of the non-target species occurring in the same ecoregion, habitat, or plant-based community as the target |
| Non-target species found in other areas or habitats likely to be invaded by the agent |
| Susceptible life stages of the non-target species and the likelihood they will be present while the agent is active |
| Non-target species’ ability to avoid attack or suppress the development of parasitoid eggs or larvae |
| Likelihood of the non-target species avoiding the agent while in diapause |
| Likelihood of the non-target species competing with the agent for food |
| Likelihood of the non-target species becoming a predator of the agent |

## References

Anon. 1996. Hazardous Substances and New Organisms Act 1996. New Zealand Government, Wellington.

Barratt, B. I. P., J. H. Todd, E. P. J. Burgess, and L. A. Malone. 2010. Developing biosafety risk hypotheses for invertebrates exposed to GM plants using conceptual food webs: a case study with elevated triacylglyceride levels in ryegrass. Environ. Biosaf. Res. 9: 163–179.

Berndt, L. A., T. M. Withers, S. Mansfield, and R. J. B. Hoare. 2009. Non-target species selection for host range testing of *Cotesia urabae*. N. Z. Plant Protect. 62: 168–173.

Bourchier, R. S. 2003. Receptor characterization of nontarget butterflies for risk assessment of biological control with the egg parasitoid *Trichogramma minutum* (Hymenoptera: Trichogrammatidae). Can. Entomol. 135: 449–466.

Kuhlmann, U., U. Schaffner, and P. G. Mason. 2005. Selection of non-target species for host specificity testing of entomophagous biological control agents, pp. 566–583 *In* M. Hoddle (ed.), Second International Symposium on Biological Control of Arthropods, Davos, Switzerland. United States Department of Agriculture, Forest Service, Morgantown, West Virginia, USA, FHTET-2005-08.

Kuhlmann, U., U. Schaffner, and P. G. Mason. 2006. Selection of non-target species for host specificity testing, pp. 15–37 *In* F. Bigler, D. Babendreier and U. Kuhlmann (eds.), Environmental Impact of Invertebrates for Biological Control of Arthropods: Methods and Risk Assessment. CABI Publishing, Wallingford, UK.

Malone, L. A., J. H. Todd, E. P. J. Burgess, C. Walter, A. Wagner, and B. I. P. Barratt. 2010. Developing risk hypotheses and selecting species for assessing non-target impacts of GM trees with novel traits: the case of altered-lignin pine trees. Environ. Biosaf. Res. 9: 181–198.

Todd, J. H., P. Ramankutty, E. I. Barraclough, and L. A. Malone. 2008. A screening model for optimising the selection of non-target species for testing the biosafety of transgenic crops. Environ. Biosaf. Res. 7: 35–56.

Toepfer, S., F. Zhang, and U. Kuhlmann. 2009. Assessing host specificity of a classical biological control agent against western corn rootworm with a recently developed testing protocol. Biol. Control 51: 26–33.

USEPA. 1998. Guidelines for Ecological Risk Assessment (EPA/630/R095/002F). United States Environmental Protection Agency, Washington, DC, USA.

Van Driesche, R. G., and M. Hoddle. 1997. Should arthropod parasitoids and predators be subject to host range testing when used as biological control agents? Agr. Hum. Values 14: 211–226.

Van Driesche, R. G., and R. Reardon (eds.). 2004. Assessing host ranges for parasitoids and predators used for classical biological control: A guide to best practice. United States Department of Agriculture, Forest Service, Morgantown, West Virginia, USA, FHTET 2004-03.

van Lenteren, J. C., D. Babendreier, F. Bigler, G. Burgio, H. M. T. Hokkanen, S. Kuske, A. J. M. Loomans, I. Menzler-Hokkanen, P. C. J. Van Rijn, M. B. Thomas, M. G. Tommasini, and Q. Q. Zeng. 2003. Environmental risk assessment of exotic natural enemies used in inundative biological control. BioControl 48: 3–38.

# Progress and problems in host specificity testing of arthropod biological control agents

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Knowledge of the host specificity of a biological control agent is essential to assess whether non-target native species may be harmed after an agent is introduced to areas outside its native range. In the 1980's, publications (e.g. Howarth 1983) on the negative impact of generalist biological control agents released in the past kicked off a controversial discussion on the environmental safety of biological control. To appease the critics and to address concerns regarding arthropod biological control, new guidelines and methods for host-range testing of biological control agents were developed in the early 2000's (Van Driesche and Reardon 2004; Bigler et al. 2006; van Lenteren et al. 2006). Being aware of potential negative effects, nowadays risk assessment for non-target impact has become one of the key elements of agent selection and it is generally recommended that potential agents should demonstrate high host specificity. Although research within the last ten years led to a greater understanding of risk associated with biological control agents, the debate is still ongoing, and biological control is still facing adverse criticism.

We investigated how the new guidelines prove themselves in practice using *Trichomalus perfectus* (Walker) (Hymenoptera: Braconidae) as a case study. The European species *T. perfectus* was identified as a key species for biological control of cabbage seedpod weevil *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), a common pest of canola, *Brassica napus* L. and *B. rapa* L. (Brassicaceae) introduced into North America from Europe in the 1930s. The ecto-parasitoid attacks the late instar larvae of its host, which feed inside canola pods. The following steps were conducted to assess the potential risk associated with a release of *T. perfectus* in North America:

1) Approximately, 50 species belonging to the genus *Ceutorhynchus* occur in central Europe and 32 in North America and thus, the number of potential non-target hosts of *T. perfectus* is immense. A step-wise process, based on what is known on the parasitoid biology available from the literature, taxonomic and phylogenetic relationships and sympatry of target and non-target species and therefore the types of potential hosts the agent is most likely to encounter, is recommended to select species for use in host specificity tests (Kuhlmann et al. 2006). Accordingly, 17 and 8 representative test species for Europe and North America were selected, respectively. A proposed non-target test list (Mason et al. 2008) was sent to regulatory authorities in Canada and feedback provided some guidance for conducting testing.

2) Small arena laboratory no-choice tests were carried out to investigate the acceptance and suitability of non-target hosts. The aim of laboratory tests on host specificity is to predict the potential ecological host range of the control agent in the area of release. Individual females were either exposed to larvae of the target *C. obstrictus* or any of the non-targets for 24 h. If females did not attack the offered target or non-target hosts within the first 24 h, the larvae were removed and females were subsequently exposed to larvae of the target, *C. obstrictus*, for another 24 hours. Results demonstrated that 5 out of the 11 European non-target species tested were frequently attacked and suitable for *T. perfectus* development. This included closely

related species from the genus *Ceutorhynchus* feeding on Brassica seed pods, stems, and stem galls. Of the four North American *Ceutorhynchus* spp. tested, seed feeders were most heavily attacked. A fifth seed-feeding species in a different genus was attacked when removed from its pod but not when enclosed as in nature. Additional experiments on the effects of non-target hosts on sex ratios and size of *T. perfectus* further showed that the attack of non-target species smaller than the target *C. obstrictus* resulted in smaller off spring and a strongly male-biased sex ratio.

3) Although choice tests are recommended after non-targets were attacked in no-choice tests, we were not able to conduct choice test due to the fact that most non-target test species would only slightly overlap with the occurrence of *C. obstrictus* in the field. Field tests may help eliminate some of the effects of the laboratory environment; however, quarantine considerations prevent these tests from being performed in the area of introduction. In order to prove the validity of our laboratory test results, we investigated the ecological host range of *T. perfectus* in its native range instead of conducting laboratory choice tests or field cage tests. In particular, we were interested in whether the 5 species that were frequently attacked by *T. perfectus* in the laboratory would be attacked in the field and to what extent. The results of our field studies confirmed that at least four of the species attacked in the laboratory are also attacked in the field. Average parasitism of these non-targets varied strongly with sites, but was generally low (1-14%).

In conclusion, the method of selecting non-targets as currently recommended is certainly a useful tool, but strongly depends on the information available. Our example showed that in reality biological control practioners are often confronted with the following problems: knowledge of non-target ecology is extremely limited; rearing methods for non-target species and the biological control agent are unknown; the parasitoid complexes of non-targets are widely unknown; the potential biological control agent can be confused with other species in the old literature, resulting in unreliable host records.

Our field and laboratory data from Europe and North America suggest that non-target species are likely to be attacked, but European field data also suggest that the impact on non-target populations is likely very low. Contradictive levels of parasitism in the laboratory and field, as shown in our example, are not uncommon, but make the assessment of risk more difficult. Current guidelines still lack suggestions on how to transform laboratory and field data into a reliable risk analysis and in most cases the interpretation of the test data remain somehow subjective. We believe that more studies on the impact on non-target species at the population level are needed (Barratt et al. 2010) and test data need to be integrated into population models to show that some level of non-target attack does not necessarily mean that a non-target is at risk. Otherwise, a zero-risk approach, e.g. no attack of test species under laboratory and field conditions, might lead to the exclusion of promising biological control agents in the future.

## References

Barratt, B.I.P., F.G. Howarth, T.M. Withers, J.M. Kean, and G.S. Ridley. 2010. Progress in risk assessment for classical biological control. Biol. Control 52: 245–254.

Bigler, F., D. Babendreier, and U. Kuhlmann. 2006. Environmental impact of invertebrates for biological control of arthropods: methods and risk assessment. CABI Publishing, Wallingford, UK.

Howarth, F.G. 1983. Classical biological control: panacea or Pandora’s box. Proc. Hawaiian Entomol. Soc. 24: 239–244.

Kuhlmann, U., U. Schaffner, and P. Mason. 2006. Selection of non-target species for host specificty testing. pp. 15–37 *In* Bigler, F., D. Babendreier, and U. Kuhlmann (eds). Environmental impact of invertebrates for biological control of arthropods: methods and risk assessment. CABI Publishing, Wallingford, UK.

Mason, P.G., D.R. Gillespie, U. Kuhlmann, T. Haye and L.Dosdall. 2008. Proposed test list for candidate biological control agents for cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) [Coleoptera: Curculionidae]. Agriculture and Agri-Food Canada, Ottawa, Ontario CANADA.

Shaw, M.R. 1994. Parasitoid host ranges. pp. 111–144 *In* Hawkins, B.A. and W. Sheehan, W. (eds.) Parasitoid Community Ecology. Oxford University Press. Oxford, UK.

Van Driesche, R.G. and R. Reardon. 2004. Assessing host ranges for parasitoids and predators used for classical biological control: a guide to best practice. United States Department of Agriculture, Forest Health Technology Enterprise Team, Morgantown, USA, FHTET 2004-03.

van Lenteren, J.C., J. Bale, F. Bigler, H.M.T. Hokkanen, and A.J.M. Loomans. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. Annu. Rev. Entomol. 51: 609–634.

# Molecular diagnostics in biological control: evaluating trophic interactions and non-target effects of insect parasitoids

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The utility of molecular tools in arthropod biological control programmes has been recognized for several years (Symondson 2002; MacDonald and Loxdale 2004; Greenstone et al. 2006; Gariepy et al. 2007). Although initially used for clarification of taxonomic and phylogenetic relationships (particularly among the parasitic Hymenoptera), molecular methods have since been applied to investigate the population genetic structure of natural enemies, quantify parasitism levels, track the spread and dispersal of introduced agents, and to assess the genetic variability of candidate agents.

Molecular diagnostics can also play a valuable role in the identification of trophic interactions, many of which remain unseen or undetected using conventional rearing and dissection methods. Knowledge of these interactions can be particularly pertinent in the evaluation of direct and indirect non-target effects in a field setting, where key players in a host-parasitoid relationship may be unknown, unanticipated, or unexpected.

Here, the utility of molecular diagnostic tools is presented in the context of parasitoids used in arthropod biological control programmes, in particular as applied to the evaluation of host-specificity of candidate agents and in the assessment of direct and indirect non-target effects. Examples of past, present, and future applications of DNA-based techniques in arthropod biological control programmes are discussed.

Cryptic species diversity has been known to influence host-specificity. Morphological and molecular studies on natural enemies previously considered to be extreme generalists have shown the existence of cryptic species that are morphologically identical, but which differ genetically and ecologically (Smith et al. 2006; Hernandez-Lopez 2011). In such a situation, one member of an alleged species complex may have a particular “niche” that may make it a more suitable agent for a given target pest or for a given set of environmental conditions. Knowledge of the genetic variability and existence of more specialized cryptic species has the potential to fine-tune natural enemy selection, enhance agent establishment, and minimize non-target impacts.

Traditional methods for evaluating the ecological host range of an agent suffer from a number of complications. The collection of individuals from the field and rearing these individuals in the laboratory to obtain and identify parasitoid adults requires a set of well-developed rearing protocols. Some insects are more amenable to laboratory rearing than others, and the inability to successfully rear target and non-target insects can preclude thorough documentation of the associated parasitoid community and the occurrence of non-target parasitism. This can be compounded by increased potential of premature, parasitoid-induced mortality in non-target hosts that die before the parasitoid can complete development to an identifiable life stage. Host dissection following collection can be used as an alternative to host rearing to detect parasitized individuals. However, morphological similarity of immature stages of closely-related parasitoid species generally precludes identification at the species level, and therefore fails to define species-specific host-parasitoid associations. As an alternative or supplement to traditional rearing and dissection methods, molecular diagnostic tools can be used to screen field-collected target and non-target hosts for the presence of candidate agents or to identify dissected immature stages (Gariepy et al. 2008a, b).

This limits the number of hosts that require rearing, and allows information to be obtained from each and every individual collected, without loss of information from host or parasitoid mortality incurred in the rearing process. When applied to ecological host range surveys, molecular identification of immature parasitoids (either within the host or dissected from the host) can provide an accurate and thorough assessment of the parasitoid community associated with a set of potential host species in the field, and provides information on new or unexpected species that may not survive the rearing process. It is important to note that detection of a parasitoid within a host does not necessarily indicate the ability of the parasitoid to complete development and kill a host, it merely indicates that a parasitism event occurred and parasitoid DNA is present. As such, molecular diagnostics may over-estimate the host range of an agent and results should be interpreted with caution, as attack does not necessarily lead to mortality. In such cases, the detection of parasitoid DNA serves as an indicator that the parasitoid may recognize the insect as a potential host, and this information can be used to refine the non-target test list and guide further laboratory or rearing experiments to determine the ability of a candidate agent to develop and/or inflict mortality on a given insect of concern.

In addition to detecting a natural enemy within an insect host, molecular diagnostic tools can be used to detect interactions between natural enemies which share a common host, and can thus be used to predict the potential for indirect non-target effects due to competition between agents. In situations where a host can only support the development of one species (in the case of gregarious parasitoids) or one individual (in the case of solitary parasitoids), the attack of this same host by another parasitoid leads to the death of one of these species by the superior competitor. The winner of the competition completes development and emerges from the host when reared; however, evidence of the inferior competitor is destroyed in the process and cannot be detected by conventional means. This is of concern if competition exists between an introduced agent and a native agent OR between multiple introduced agents, as competitive displacement resulting from multiparasitism is difficult to confirm and can go undetected by traditional rearing and dissection methods. In a scenario where multiple agents (introduced and/or native) attack the same target pest, DNA from specimens from laboratory experiments and/or field-collected samples could be screened with molecular markers to detect the simultaneous occurrence of different parasitoids within an individual host (Gariepy et al. 2008a; Traugott et al. 2008; Gariepy and Messing 2012). In this context, the results could indicate interspecific competition between multiple agents that share the same targeted host and which may impede successful biological control of the targeted pest or pose a threat to native natural enemies. Knowledge of these interactions may be used to decide between one or more agents to maximize compatibility and minimize indirect non-target effects (i.e., which agent or agents are less likely to competitively exclude additional or existing agents).

A similar situation is encountered when dealing with hyperparasitoids from field-collected hosts. Hyperparasitoids often have a fairly broad host range, and in the rearing process a hyperparasitized individual yields only a hyperparasitoid adult, and evidence of the primary parasitoid species that initially attacked the host insect is lost. The potential negative impact of hyperparasitoids on biological control programs is often debated (Sullivan 1987; Schooler et al. 2011). However, more detailed knowledge regarding the host range, preferences, and impact of hyperparasitoids may help clarify their role in the food web and determine whether they are likely to have disruptive effects in a given biological control program (Gariepy and Messing 2012). Molecular diagnostic tools, including multiplex PCR assays, have the potential to detect several key players from different trophic levels simultaneously within an insect host, thereby allowing the identification of both primary and secondary parasitoids. This uncovers valuable information pertaining to host-parasitoid-hyperparasitoid interactions from field-collected material in a way that was not achievable in the past, and which can uncover previously unknown trophic links (Traugott et al. 2008; Gariepy and Messing 2012). Again, it is important to note that results must be interpreted with caution; the detection of DNA from the key players indicates their occurrence at a given point in time and does not necessarily indicate their survival or fitness as a result of the interaction. Furthermore, certain interactions may go undetected depending on the state of one or more of the players leading to an incomplete picture of the trophic interactions. For example, there may be a narrow window of time in which both the hyper- and primary parasitoids are detectable simultaneously within a host because as the hyperparasitoid consumes and digests the primary parasitoid, less and less DNA of the primary parasitoid is available for detection. If this is the case, then a hyperparasitoid is detected, but the primary parasitoid information is lost in much the same way as in reared specimens. Although this is less of an issue in DNA-based analysis of specimens from a laboratory experiment where timing of molecular analysis can be modified to capture the event within this window of opportunity, results from field-collected specimens may be somewhat ambiguous and difficult to interpret. However, as DNA-based techniques and methodology improve over time, more sensitive approaches for detection are likely to resolve some of the key issues encountered in the teasing out of trophic interactions of natural enemies used in arthropod biological control programmes.

## References

Gariepy, T.D., U. Kuhlmann, C. Gillott, and M. Erlandson. 2007. Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of arthropods. J. Appl. Entomol. 131: 225−240.

Gariepy, T.D., U. Kuhlmann, C. Gillott, and M. Erlandson. 2008a. A large-scale comparison of conventional and molecular methods for the evaluation of host-parasitoid associations in non-target risk assessment studies. J. Appl. Ecol. 45: 708−715.

Gariepy, T.D., A.B.Broadbent, S. Ethier, U. Kuhlmann, C. Gillott, and M. Erlandson. 2008b. Detection of European *Peristenus digoneutis* Loan in mirid populations in southern Ontario. Biocontrol Sci. Technol. 8: 583−590.

Gariepy, T.D., and R.H. Messing. 2012. Development and use of molecular diagnostic tools to determine trophic links and interspecific interactions in aphid-parasitoid communities in Hawaii. Biol. Control: 26−38.

Greenstone, M.H. 2006. Molecular methods for assessing parasitism. Bull. Entomol. Res. 96: 1−13.

Hernandez-Lopez, A., R. Rougerie, S. Augustin, C. Lees, R. Tomov, M. Kenis, E. Cota, E. Kullaj, C. Hansson, G. Grabenweger, A. Roques, and C. Lopez-Vaamonde. 2012. Host tracking or cryptic adaptation? Phylogeography of *Pediobius saulius* (Hymenoptera, Eulophidae), a parasitoid of the highly invasive horse-chestnut leaf miner. Evol. Appl. 5: 256−269.

MacDonald, C., and H. Loxdale. 2004. Molecular markers to study population structure and dynamics in beneficial insects (parasitoids and predators). Int. J. Pest Manage. 50: 215−224.

Schooler, S.S., P. DeBarro, and A.R. Ives. 2011. The potential for hyperparasitism to compromise biological control: Why don't hyperparasitoids drive their primary parasitoid hosts extinct? Biol. Control 58: 167−173.

Smith, M.A., N.E. Woodley, D.H. Janzen, W. Hallwachs, and P.D. Hebert. 2006. DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). P. Natl. Acad. Sci. USA 103: 3657−3662.

Sullivan, D.J. 1987. Insect hyperparasitism. Annu. Rev. Entomol. 32: 49−70.

Symondson, W.O.C. 2002. Molecular identification of prey in predator diets. Mol. Ecol. 11: 627−641.

Traugott, M., J.R. Bell, G.R. Broad, W. Powell, F.J.F. vanVeen, I.M.G. Vollhardt, and W.O.C. Symondson. 2008. Endoparasitism in cereal aphids: Molecular analysis of a whole parasitoid community. Mol. Ecol. 17: 3928−3938.

# Natural host range of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) in Morocco: could it help predict host range in new areas?

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Information on natural host range of proposed biological control agents can assist researchers to predict potential host range in new areas of introduction (e.g. Haye et al. 2005). Also, regulators responsible for approvals for new biological control agent introductions often request information about host range in the country of origin and in other areas where the same organism has been introduced (e.g. Food and Agriculture Organisation 1996).

*Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) is a solitary, koinobiont endoparasitoid of the adult stage of its host and a parasitoid population from the Mediterranean region was introduced into Australia in 1977 (Aeschlimann 1983; Cullen and Hopkins 1982) and then into New Zealand from Australia in 1982 (Stufkens et al. 1987) for biological control of *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae), an introduced pest of lucerne. Yanco Agricultural Institute in New South Wales was the location of one of the earliest releases in Australia. In New Zealand, the parasitoid was released after limited host-specificity testing, as allowed by regulation at the time. Since then 19 non-target weevil species have been recorded in the field as hosts of *M. aethiopoides*, and 12 of the 14 native species are members of the subfamily Entiminae, tribe Leptopiini (Barratt et al. 2012). Recent molecular studies suggest that the *M. aethiopoides* population introduced into New Zealand was sourced from an Australian population originating from Morocco (Vink et al. 2003). Phillips et al. (2008) proposed the existence of two sympatric biotypes of *M. aethiopoides*, one associated with *Sitona* and the other with *Hypera* (Curculionidae: Hyperinae).

This study set out to determine, retrospectively, whether better knowledge of the natural host range of *M. aethiopoides* in Morocco and determination of the novel host-range in Australia would have helped predict the host range of *M. aethiopoides* in New Zealand. Field surveys of the weevil fauna were carried out in both Morocco and in Australia to identify those species that are included within the natural and novel host ranges, respectively, and hence to determine the taxonomic breadth of potential host species that may be available to *M. aethiopoides*. In Australia, previous work detected a single incidence of parasitism of the native weevil '*Prosayleus'* sp. 2 by *M. aethiopoides* (Barratt et al. 2005) so collecting aimed to investigate further the scale and phenology of this association, as well as to detect other non-target hosts. Parasitism of the target host *S. discoideus*, and phenology of the parasitoid were investigated so that impacts on nontarget species can be better understood in relation to seasonal target host availability.

Sampling was conducted in lucerne-growing areas of three regions of Morocco over nine months of the year at sites near Rabat, Marrakech and Ar-Rachidia, the latter being a major source of the *M. aethiopoides* populations imported into Australia (Aeschlimann 1983). At Yanco samples were taken from both dryland and irrigated lucerne. Weevils were preserved and shipped to New Zealand for dissection to determine parasitism, and specimens from Morocco and Australia were identified as far as possible to species. Most parasitoids from the non-target host weevil species found, and a sample of those from *S. discoideus* was analysed by extracting, amplifying and sequencing a fragment of the mitochondrial gene CO1 (Vink et al. 2012).

Over 3,600 weevils were collected in Morocco, of which 84% were *S. discoideus*, and 46 other species were identified. Other than *S. discoideus*, the most commonly collected weevil species was *Hypera postica* (Gyllenhal) (Curculionidae: Hyperinae). Other common species were *Lixus ulcerosus* Petri (Curculionidae: Lixinae) and *Malvapion malvae* (Fabricius) (Brentidae: Apioninae) at Ar-Rachidia and Marrakech regions; *Charagmus gressorius* (F.) (Entiminae: Sitonini) was common near Rabat.

Less than 10% of *S. discoideus* collected across all three regions was parasite, and 13 individuals of four other species were parasitised; *C. gressorius*, *C. griseus* (F.), *H. postica* and *Aulacobaris fallax* (Brisout). Sequencing of the COI gene suggested that parasitoids from all but *A. fallax* were *M. aethiopoides*. In general, parasitism of weevil species other than *S. discoideus* coincided with the period when numbers and parasitism of *S. discoideus* were at their lowest and when many of the other species were most abundant. The known natural host range for *M. aethiopoides* from this and previous studies is shown in Table 1.

Table 1: The known hosts of *M. aethiopoides* in its natural geographical range

| Genus species | Subfamily: Tribe | Genus species | Subfamily: Tribe |
|---|---|---|---|
| *Charagmus griseus** | Entiminae: Sitonini | *Sitona puncticollis* | Entiminae: Sitonini |
| *Charagmus gressorius** | " | *Sitona sulcifrons* | " |
| *Sitona discoideus* | " | *Sitona tenuis* | " |
| *Sitona hispidulus* | " | *Hypera meles* | Hyperinae: Hyperini |
| *Sitona lepidus* | " | *Hypera nigrirostris* | " |
| *Sitona lineatus* | " | *Hypera postica* | " |
| *Sitona macularius* | " | | |

* new host records from this study

In Australia, of 3,338 weevils collected at Yanco >93% were *S. discoideus*, and 13 other weevil species were identified. The native Australian weevil '*Prosayleus*' sp. 2 was the most commonly collected species followed by *Ethemaia sellata* Pascoe (Curculionidae: Cyclominae), both almost exclusively found in dryland lucerne. The seasonality of *S. discoideus* observed in irrigated and dryland lucerne at Yanco appeared to be quite different than in Morocco, with a more distinct autumn/winter peak in dryland lucerne. In irrigated lucerne, *S. discoideus* was collected in all months, with peak numbers occurring in late spring and a small winter peak.

Less than 25% of *S. discoideus* adults collected in dryland lucerne were parasitised, and this proportion was highest in October. In irrigated lucerne, parasitism levels were low throughout the season, with the greatest proportion (16%) being found parasitised in September. No non-target species were parasitised.

This study addressed the question of whether information on natural host range in Morocco and novel host range in Australia could have assisted regulators in New Zealand to make a decision on the likely environmental risk posed by the introduction of *M. aethiopoides* for control of *S. discoideus*. The context for the study was the knowledge that in New Zealand this parasitoid attacks at least 19 non-target weevils in the field.

Earlier work by Loan (1975) and Aeschlimann (1980) recorded the known host range of *M. aethiopoides* as comprising eight *Sitona* and three *Hypera* species. In this study we have added another two host species in the genus *Charagmus*. This genus was previously considered as a subgenus of *Sitona* but now has full genus status (Velazquez De Castro et al. 2007).

The results of this study suggest that members of the subfamily Entiminae are acceptable hosts for the *Sitona*-associated biotype of *M. aethiopoides*. The tribe Sitonini is generally separated from the bulk of Entiminae (e.g. Marvaldi 1997; Marvaldi et al. in press), but its precise status and relationship with other entimine tribes remains to be established. The tribe Sitonini does not occur naturally in Australia and New Zealand. In these countries the entimines mainly belong to the large, southern hemisphere tribe Leptopiini that is only poorly represented (if at all) in the northern hemisphere, where Sitonini are most diverse. The small northern tribe Tropiphorini (= Alophini), sometimes grouped together with Leptopiini (Alonso-Zarazaga and Lyal 1999) is also considered to be different from the bulk of Entiminae. Recent phylogenetic analyses of molecular data have established that Hyperinae and Entiminae are closely related. Hundsdörfer et al. (2009) found a sister-group relationship between Hyperinae and Sitonini, whereas McKenna et al. (2009) found *Hypera* nested within Entiminae, and *Sitona* grouping with genera of the subfamily Cyclominae. However, a comprehensive molecular analysis of Australian weevils (Gunter and Oberprieler, unpublished) found Hyperinae clustering as the sister-group of Entiminae. While the exact relationship between Hyperinae and Entiminae remains uncertain, it is evident that the natural host range of *M. aethiopoides* reflects a largely phylogenetic pattern.

If a decision about the introduction of *M. aethiopoides* into New Zealand was being made today under current regulations (HSNO Act 1996), and using information available from this study, a risk assessment would take into account information on the natural host range in Morocco and host range in other new areas of introduction e.g. Australia. Given that there are no members of the tribes Sitonini or Hyperini in New Zealand, and that these were the only tribes found to be hosts of *M. aethiopoides* in Morocco, it could be argued that no host range testing should be required. However, the combination of evidence that: 1) in Australia a native species of *Prosayleus* (Leptopiini) was found to be a host, 2) in New Zealand Leptopiini are well represented, and 3) no Leptopiini were present among the Moroccan weevils surveyed (they are scarcely, if at all represented there), was sufficient to indicate that host range testing should be conducted with native New Zealand Leptopiini. Had this been done pre-release of *M. aethiopoides*, it is likely that non-target parasitism would have been detected and possible that an application to release might have been declined.

Current knowledge of weevil classification is advanced from that available when *M. aethiopoides* was introduced into Australia and New Zealand. The relationship between Entiminae and Hyperinae was at that time considered more distant than it is now, which would have suggested a wider host range of the parasitoid, and hence a greater risk for potential non-target effects than would be predicted now based on the indicated closer relationship between these two subfamilies. We conclude that establishing native host range of proposed biological control agents is valuable, as is knowledge of host range in other areas of introduction. Furthermore, thorough host range testing in the proposed new area of introduction is critical, especially if the taxonomic composition of the potential host fauna differs from that in the native range. Host phylogeny is an important consideration in risk assessment, but in a large and complex family such as Curculionidae, in which phylogenetic relationships among subfamilies and tribes are still poorly understood, new taxonomic and phylogenetic studies can change existing classification. Complete reliance on current classification schemes in this context may therefore be misleading.

## References

Aeschlimann, J. P. 1980. The *Sitona* (Col.: Curculionidae) species occurring on *Medicago* and their natural enemies in the Mediterranean region. Entomophaga 25: 139–153.

Aeschlimann, J. P. 1983. Sources of importation, establishment and spread in Australia of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae), a parasitoid of *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae). J. Aust. Entomol. Soc. 22: 325–331.

Alonso-Zarazaga, M. A. and C. H. C. Lyal (eds). 1999. A world catalogue of families and genera of Curculionoidea (Insecta: Coleoptera). Entomopraxis, Barcelona, Spain.

Barratt, B. I. P., R. G. Oberprieler, D. Barton, M. Mouna, M. Stevens, M. A. Alonso-Zarazaga, C. J. Vink, and C. M. Ferguson. 2012. Could research in the native range, and non-target host range in Australia, have helped predict host range of the parasitoid *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae), a biological control agent introduced for *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) in New Zealand? BioControl 57: 735–750.

Barratt, B. I. P., R. G. Oberprieler, C. M. Ferguson, and S. Hardwick. 2005. Parasitism of the lucerne pest *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) and non-target weevils by *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) in south-eastern Australia, with an assessment of the taxonomic affinities of non-target hosts of *M. aethiopoides* recorded from Australia and New Zealand. Aust. J. Entomol. 44: 192–200.

Cullen, J. M., and D. C. Hopkins. 1982. Rearing, release and recovery of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) imported for the control of *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) in south eastern Australia. J. Aust. Entomol. Soc. 21: 279–284.

Food and Agriculture Organisation, 1996. Code of conduct for the import and release of exotic biological control agents. Food and Agriculture Organisation, Rome, Italy.

Haye, T., H. Goulet, P. G. Mason, and U. Kuhlmann. 2005. Does fundamental host range match ecological host range? A retrospective case study of a *Lygus* plant bug parasitoid. Biol. Control 35: 55–67.

Hundsdörfer, A. K., J. Rheinheimer, and M. Wink. 2009. Towards the phylogeny of the Curculionoidea (Coleoptera): reconstructions from mitochondrial and nuclear ribosomal DNA sequences. Zool. Anz. 248: 9–31.

Loan, C. C. 1975. A review of Haliday species of *Microctonus* (Hym. : Braconidae, Euphorinae). Entomophaga 20: 31–41.

Marvaldi, A. E. 1997. Higher level phylogeny of Curculionidae (Coleoptera: Curculionoidea) based mainly on larval characters, with special reference to broad-nosed weevils. Cladistics 13: 285–312.

Marvaldi, A. E., A. A. Lanteri, M. G. del Río, and R. G. Oberprieler. (2013). Entiminae Schoenherr 1823, pp. *In* R. G. Beutel & R. A. B. Leschen (eds.), Handbook of Zoology Vol. IV, Part 38, Coleoptera, Beetles. Vol. 3: Morphology and Systematics (Polyphaga partim). Walter de Gruyter, Berlin, Germany. (in press).

McKenna, D. M., A. S. Sequeira, A. E. Marvaldi, and B. D. Farrell. 2009. Temporal lags and overlap in the diversification of weevils and flowering plants. P. Natl. Acad. Sci. USA 106: 7083–7088.

Phillips, C. B., C. J. Vink, A. Blanchet, and K. A. Hoelmer. 2008. Hosts are more important than destinations: what genetic variation in *Microctonus aethiopoides*

(Hymenoptera: Braconidae) means for foreign exploration for natural enemies. Mol. Phylogenet. Evol. 49: 467–476.

Stufkens, M. W., J. A. Farrell, and S. L. Goldson. 1987. Establishment of *Microctonus aethiopoides*, a parasitoid of the sitona weevil in New Zealand, pp. 31-32. *In* A. J. Popay (ed.), Proceedings of the 40th New Zealand Weed and Pest Control Conference, The New Zealand Weed and Pest Control Society.

Velazquez De Castro, A. J., M. A. Alonso-Zarazaga, and R. Outerelo. 2007. Systematics of Sitonini (Coleoptera: Curculionidae: Entiminae), with a hypothesis on the evolution of feeding habits. Syst. Entomol. 32: 312–331.

Vink, C. J., B. I. P. Barratt, C. B. Phillips, and D. M. Barton. 2012. Moroccan specimens of *Microctonus aethiopoides* spice our understanding of genetic variation in this internationally important braconid parasitoid of adult weevils. BioControl 57: 751–758.

Vink, C. J., C. B. Phillips, A. D. Mitchell, L. M. Winder, and R. P. Cane. 2003. Genetic variation in *Microctonus aethiopoides* (Hymenoptera: Braconidae). Biol. Control 28: 251–264.

# Proposed new risk-assessment strategies for the introduction of polyphagous arthropods in biological control programmes in New Zealand

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The introduction of biological control agents (BCAs) into New Zealand is regulated under the Hazardous Substances and New Organisms (HSNO) Act through the Environmental Protection Authority (EPA). Recent and potential future pest incursions, combined with the potential withdrawal of registration for organophosphate and carbamate pesticides left the crop production industry with fewer options for the management of arthropod pests. Increasing resistance in pests to insecticides presents additional problems, further restricting grower options. In response, the EPA is considering the implications of the Act for introductions of polyphagous BCAs for use in Integrated Pest Management (IPM). The HSNO Act requires consideration of five minimum standards[1] regarding the "significant" impact of BCAs on native species, the environment, human health, genetic diversity and ability to vector disease; and although seemingly explicit, interpretation of the terms "significant", "displacement" and "native" can cause confusion. For example, the subjective measure of "significant" means different things to different people. Does "displacement" refer to population reduction, and is it conceptually long term, or could it be localised? How much does this matter in the case of bacteria or native psyllids for example, some of which may become locally extinct as a result of new organism introductions, but would still maintain a viable population. Does "native" refer to naturalised, such as the silvereye, *Zosterops lateralis* Latham (Passeriformes :Zosteropidae*)*; native, such as the fantail, *Rhipidura fuliginosa* Sparrman (Passeriformes: Rhipiduridae), which is also found in eastern Australia; or endemic, such as the kiwi, *Apteryx* spp. (Apterygiformes: Apterygidae); and to what extent can "valued" species be considered, for example earthworms, which are introduced but deliver important ecosystem services?

Consideration of the risks and benefits potentially derived from an organisms' introduction come after the HSNO Act minimum standards have been met. One key concern is the impact on non-target species, which in most cases is measured through laboratory bioassays of host-specificity. The major challenge facing applications to introduce polyphagous BCAs is therefore the assessment of non-target impact and the perception verses quantification of risk. As a consequence, biological control programmes in New Zealand currently aim to avoid species that are not host-specific, yet the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), which is effective against greenhouse whitefly, *Trialeurodes vaporarium* Westwood (Hemiptera: Aleyrodidae), was introduced into New Zealand in 1934 and is now known globally to parasitise at least 15 species of whiteflies including, in the laboratory, some native

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[1] HSNO Act Minimum Standards:
An application shall be declined if the new organism is likely to-

- cause any significant displacement of any native species within its natural habitat;
- cause any significant deterioration of natural habitats;
- cause any significant adverse effects on human health and safety;
- cause any significant adverse effect to New Zealand's inherent genetic diversity; or
- cause disease, be parasitic, or become a vector for human, animal or plant disease, unless the purpose is to cause disease, be parasitic, or vector disease.

species. However, it is rarely detected outside protected cropping (Martin 1989). So, how predictable is impact and is the precautionary approach a reasonable one in terms of net benefit (for example Thomas and Willis 1998)? The EPA is developing new risk assessment strategies to try and answer these questions.

To meet the minimum standards of the HSNO Act, several tools are available. An initial literature search can be used to identify efficacy of the proposed BCA in existing international biological control programmes. For example, a recent application to the EPA for a BCA to manage the codling moth, *Lymantria dispar* (L.) (Lepidoptera: Tortricidae), provided background information on the efficacy, biology and phenology of the proposed BCA (Kuhlmann and Mills 1999; Bezemer and Mills 2002; Mills 2005; Jumean et al. 2009).

In addition to the literature search, some form of habitat matching can be used to predict the potential range of a proposed BCA, although we acknowledge that there are many other factors that also contribute to the invasive potential. CLIMEX modelling uses climatic responses of organisms and meteorological information to predict the potential distribution of a yet to be introduced exotic organism under current climate conditions. Predictions from CLIMEX models can be made for specific or multiple spatial scales, from global to local (Sutherst et al. 2004). However, CLIMEX is not the only option for modelling potential distributions of introduced species; spatial relationships between arthropods and their geographical and climatic distribution can predict the distribution of a variety of potential pests (Cocu et al. 2005; Gevrey and Worner 2006; Pitt et al. 2007). In addition to climate matching, it is important to consider other biological and ecological traits; for example, an organisms overwintering ability (Hatherly et al. 2005), the presence of empty niche space suitable for the introduced organism, low inter-specific competition and readily available food sources. However, Vennette et al. (2010) made the important point that diverse methods of mapping risk based on climate or habitat variability have the potential to yield different maps representing a different level of risk for new BCAs depending on the parameters used to create the map (Vennette et al. 2010). Thus, regulators must decide how to incorporate the various risk parameters into the decision-making process, and they must be clear that they understand what the different risk maps portray.

No-choice host range testing has traditionally been used to establish the risks of a BCA to native and valued species. It is particularly valuable in assessing the risks to native plants in classical weed biological control. It is however, virtually impossible to present the results of host-range testing in augmentative biological control using polyphagous arthropods, as by definition, they survive on a range of hosts. Host-range testing can identify physiological host range, but traditional host-range testing cannot predict the ecological impact on populations of native and/or valued species (Louda et al. 2003). Host-range testing information used to assess impact risks from polyphagous arthropods could include information on the ecology, phenology and behaviour of the proposed agent and susceptible hosts similar to that described by Barratt et al. (2000). Alternatively, limited choice testing can be used to test for oviposition on non-target hosts, but caution should be used as this technique can produce false-negative results (Withers and Mansfield 2005). Host range testing cannot cover all species that may be at risk as that would mean testing hundreds of potential hosts (van Lenteren 2001). We therefore suggest an initial host testing list that includes current introduced and naturally occurring biological control agents, native species that are considered to have high cultural value, are rare or endangered, and organisms that have commercial, cultural or aesthetic significance (Sands and Van Driesche 2000). However, we recognise that laboratory host testing may not yield ecologically significant results as experiments where arthropods are confined in artificial arenas overestimate the capacity of the predator and ignore avoidance or non-encounter behaviour (De Clercq 2002).

Two final points for consideration are the dispersal capacity and establishment potential of any proposed BCA. Predators with good dispersal abilities have a greater potential to invade environments outside the one intended (De Clercq 2002), and if they disperse rapidly and successfully, they may have a greater impact on native or valued species. However, rapid dispersal could limit the mating potential and therefore the survival ability of the organism (Jermy 1980).

In most cases with the release of a classical BCA, establishment is considered essential as the organism must establish self sustaining populations in order to manage pest populations. However, in the case of augmentative biological control, for example, in glasshouses, if applicants demonstrate that an organism is unlikely to establish outside controlled conditions, i.e. in the native environment, there may be space within the minimum standards to allow for the release of such polyphagous arthropods.

Environmental risks, costs and benefits associated with the release of BCAs have to be balanced with the benefits, and also include the risk of not doing anything. While host-range and the biological characteristics (e.g. phenology) of the proposed organism have typically been central to risk assessment, it is increasingly being recognised that there are additional elements that can be incorporated into the risk assessment where host-range testing is of limited value (van Lenteren et al. 2002). The EPA currently assesses risks, costs and benefits by estimating the magnitude and nature of any possible effects and their likelihood. For each effect, the combination of components determines the level of the risk associated with that effect. Lack of data often means that risks are presented as singular results, while in reality, they may be better considered as ‘families’ of data which link probability with different levels of outcome (magnitude). The magnitude of effect is described in terms of the elements that may be affected, and a suite of qualitative descriptors are used to describe the magnitude of effect and to gauge the end result.

Both risks and benefits can be economic, cultural and/or environmental and benefits can include re-establishment of ecological guilds and the food webs of which they are part. For example, pests in the natural environment may also inadvertently be affected by new predators. The tomato potato psyllid, *Bactericera cockerelli* Sulc (Hemiptera: Triozidae) (TPP), occurs on native Solonaceae in New Zealand and has displaced native psyllids in their native environment (Puketapu and Roskruge 2011). The opportunity to rebalance natural food web dynamics by introducing a predator of TPP can therefore be included as a benefit in any risk analysis.

We propose a method of identifying actual risks associated with polyphagous BCAs and distinguishing these from perceived risks. Table 1 outlines six predatory arthropod species that were introduced in to New Zealand prior to implementation of the HSNO Act. We recommend a system of habitat matching for each of these “*post hoc*”. If we treat them as future invasive pests and identify where they could establish and the impact they could have on New Zealand flora and fauna; and then identify where they established and the impact they have actually had, we can begin to identify where genuine impacts and risks from future BCAs may occur.

Table 1. Biological control agents established in New Zealand prior to regulation under the HSNO Act, and currently being used in IPM programmes.

| **Biological control Agent** | **Country of Origin** | **Established in New Zealand** |
|---|---|---|
| *Aphidius colmani* Vierek (Hymenoptera: Aphidiidae) | India[1] | before 1970[2] |
| *Encarsia formosa* Gahan (Hymenoptera: Aphidiidae) | Europe[3] | 1934[3] |
| *Hypoaspis miles* Berlese (Mesostigmata: Laelapidae) | Italy[4] | not known to be established |
| *Neoseiulus cucumeris* Oudemans (Mesostigmata: Phytoseiidae) | Northern Europe[5] | prior to regulation (N Martin pers. comm.) |
| *Phytoseiulus persimilis* Athias-Henriot (Mesostigmata: Phytoseiidae) | Mediterranean[6] | 1979[7] |
| *Orius vicinus* Ribaut (Hemiptera: Anthocoridae) | Europe[8] | 1991[8] |

[1](Stary 1972); [2](Valentine 1967); [3](Martin 1989); [4](Walter and Campbell 2003); [5](De Klerk and Ramakers 1986); [6](Kennett and Caltagirone 1968); [7](Walker et al. 1981); [8](Lariviere and Wearing 1994).

In conclusion, despite the advantages of being able to consider benefits in New Zealand, there is still a tendency to be “risk averse”. While there are a number of theoretical pathways that could be used to address the minimum standards, there is consensus amongst biological control practitioners that there are still too many unknown and undefined risks associated with the release of a generalist predator for biological control of arthropods. Consequently, we are interested in devising a programme for future research that would go some way to defining the unknown risks and quantifying the genuine impacts a generalist arthropod might have in New Zealand.

## References

Barratt, B., C. Ferguson, S. Goldson, C. Phillips, and D. Hannah. 2000. Predicting the risk from biological control agent introductions: A New Zealand approach. *In* P. Follett and J. Duan (eds.), Nontarget effects of biological control. Kluwer Academic Publishers, Norwell, MA, USA.

Bezemer, T., and N. Mills. 2002. Clutch size decisions of a gregarious parasitoid under laboratory and field conditions. Anim. Behav. 66: 1119-1128.

Cocu, N., R. Harrington, M. Rounsevell, S. Worner, and M. Hulle. 2005. Geographical location, climate and land use influences on the phenology and numbers of the aphid, *Myzus persicae*, in Europe. J. Biogeog. 32: 615-632.

De Clercq, P. 2002. Dark clouds and their silver linings: Exotic generalist predators in augmentative biological control. Neotrop. Entomol. 31: 169-176.

De Klerk, M., and P. Ramakers. 1986. Monitoring population densities of the phytoseiid predator *Amblyseius cucumeris* and its prey after large scale introductions to control *Thrips tabaci* on sweet pepper. Internationaal Symposium over Fytofarmacie en Fytiatrie 38: 1045-1048.

Gevrey, M., and S. Worner. 2006. Prediction of global distribution of insect pest species in relation to climate by using an ecological infomatics method. J. Econ. Entomol. 99: 979-986.

Hatherly, I., A. Hart, A. Tullett, and J. Bale. 2005. Use of thermal data as a screen for the establishment potential of non-native biological control agents in the UK. BioControl 50: 687-698.

Jermy, T. 1980. The introduction of *Perillus bioculatus* into Europe to control the Colorado beetle. OEPP/EPPO Bulletin 10: 475-479.

Jumean, Z., E. Jones, and G. Gries. 2009. Does aggregation behaviour of codling moth larvae, *Cydia pomonella*, increase the risk of parasitism by *Mastrus ridibundus*? Biol. Control 49: 254-258.

Kennett, C., and L. Caltagirone. 1968. Biosystematics of *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae). Acarologia 10: 563-577.

Kuhlmann, U., and N. Mills. 1999. Exploring the biodiversity of Central Asia to assess specialized parasitoids for biological control of apple pests in Europe and North America. Integrated Plant Protection in Orchards; IOBC wprs Bulletin 22: 1-6.

Lariviere, M., and C. Wearing. 1994. *Orius vicinus* (Ribaut) (Heteroptera: Anthocoridae), a predator of orchard pests new to New Zealand. New Zeal. Entomol. 17: 17-21.

Louda, S., A. Arnett, T. Rand, and F. Russell. 2003. Invasiveness of some biological control insects and adequacy of their ecological risk assessment and regulation. Conservation Biology 17: 73-82.

Martin, N. 1989. *Trialeurodes vaparariorum* (Westwood), greenhouse whitefly (Homoptera: Aleyrodidae), pp. 251-254. *In* P. J. Cameron, R. L. Hill, J. Bain and W. P. Thomas (eds.), A review of biological control of invertebrate pests and weeds in New Zealand 1874-1987. CAB International, Wallingford, Oxon, UK.

Mills, N. 2005. Selecting effective parasitoids for biological control introductions: Codling moth case study. Biol. Control 34: 274-282.

Pitt, J., J. Regniere, and S. Worner. 2007. Risk assessment of the gypsy moth, *Lymantria dispar* (L), in New Zealand based on phenology modelling. Int. J. Biometerol. 51: 295-305.

Puketapu, A., and N. Roskruge. 2011. The tomato-potato psyllid lifecycle on three traditional Maori food sources. Agron. New Zeal. 41: 167-173.

Sands, D., and R. Van Dreische. 2000. Evaluating the host range of agents for biological control of arthropods: rationale, methodology and interpretation, pp. 69–83 *In* R. Van Driesche, T.A. Heard, A.S. McClay, R. Reardon (eds.), Proceedings of Session: Host Specificity Testing of Exotic Arthropod Biological Control Arthropod Biological Control Agents - The Biological Basis for Improvement in Safety, USDA Forest Service, Publication #FHTET-99-1, August 2000, Morgantown, WV, USA.

Stary, P. 1972. *Apidius platensis* Brethes, its distribution and host range (Hymenoptera, Aphidiidae). Orient. Insects 6: 359–370.

Sutherst, R., G. Maywald, W. Bottomley, and A. Bourne 2004. CLIMEX v2, CD and user's guide computer program, version By Sutherst, R., G. Maywald, W. Bottomley, and A. Bourne, Melbourne, Australia.

Thomas, M., and A. Willis. 1998. Biocontrol-risky but necessary? Trends Ecol. Evol. 13: 325–329.

Valentine, E. 1967. Biological control of aphids. Proceedings of the 20th New Zealand Weed Pest Control Conference: 204–207.

van Lenteren, J. (ed.). 2001. Harvesting safely from biodiversity: natural enemies as sustainable and environmentally friendly solutions for pest control. pp. 15-30.*In* J.A. Lockwood, F.G. Howarth and M.F. Purcell (eds.) Balancing Nature: Assessing the Impact of Importing Non-Native Biological Control Agents, Thomas Say Publications in Entomology, Entomol. Soc. America.

van Lenteren, J., D. Babendreier, F. Bigler, G. Burgio, H. Hokkanen, S. Kuske, A. Loomans, I. Menzler-Hokkanen, P. Van Rijn, M. Thomas, M. Tommasini, and Q.-Q.

Zeng. 2002. Environmental risk assessment of exotic natural enemies used in inundative biological control. BioControl 48: 3–38.

Vennette, R., D. Kriticos, R. Magarey, F. Koch, R. Baker, S. Worner, N. Raboteaux, D. McKenney, E. Dobesberger, D. Yemshanov, D. PJ, W. Hutchison, G. Fowler, T. Kalaris, and J. Pedlar. 2010. Pest risk maps for invasive alien species: A roadmap for improvement. BioScience 60: 349–362.

Walker, J., C. Wearing, C. Proffitt, and J. Charles. 1981. Predator for two-spotted spider mite established in orchards. Orchard. New Zeal. 54: 340.

Walter, D. E., and N. J. H. Campbell. 2003. Exotic vs endemic biocontrol agents: would the real *Stratiolaelaps miles* (Berlese) (Acari: Mesostigmata: Laelapidae), please stand up? Biol. Control 26: 253–269.

Withers, T., and S. Mansfield. 2005. Choice or no-choice tests? Effects of experimental design on the expression of host range, pp. 620–633, *In* M. S. Hoddle (compiler) Second International Symposium on Biological Control of Arthropods, Davos, Switzerland, USDA Forest Service Publication FHTET-2005-08, Morgantown, WV, USA.

# Host specificity testing and quantitative risk assessment – A case study with introduction of exotic parasitoids for classical biological control of the emerald ash borer

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Host-specificity testing is a critical component of natural enemy introductions to assess the potential ecological risk of candidate biological control agents. It has long been used in classical weed biological control programs (Zwölfer and Harris 1971; Wapshere 1974). This approach is being gradually adopted in classical arthropod biological control programs to assess the potential ecological risk for insect predators and parasitoids (Van Driesche and Reardon 2004; Bigler et al. 2006). Host ranges of arthropod biological control agents derived from empirical host-specificity tests are often the primary (if not sole) data used by regulatory agencies and stake holders to evaluate the safety or risk of candidate agents (Van Driesche and Reardon 2004; Sheppard et al. 2005).

From the perspective of quantitative risk assessment, however, host ranges derived from the empirical host-specificity tests only address one aspect of the risk model, which is equivalent to the definition of “hazard” in ecotoxicology risk assessment – i.e., the potential for the risk (or non-target impact) to occur (Burgman 2005). The other critical aspect of the risk assessment model relates to the ecological context or “exposure” situation where the “hazard – i.e. potential of the non-target impact would likely occur. Unfortunately, our knowledge of the receiving ecosystem is often incomplete or lacking, and this limitation has made the formal, quantitative risk assessment for introduction of arthropod predators or parasitoids extremely difficult (if not possible).

In the present study, a case study with classical biological control of an invasive buprestid wood borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in the USA is used to illustrate a quantitative approach to assessing the potential risk of introducing the exotic parasitoid *Spathius galinae* Belokobylskij & Strazanac (Hymenoptera: Braconidae) from the pest’s area of origin (Belokobylskij et al. 2012; Duan et al. 2012). This approach involves not only selection of an appropriate list of non-target wood-boring insects for laboratory host-specificity testing, but also use of appropriately paired positive (target) controls so that a relative host-specificity hazard index can be calculated for the candidate agent. The test list of non-target species of woodborers in North America was selected based on both phylogenetic affinities between the target pest and non-target species and ecological affinities between indigenous Coleoptera and the target pest (Table 1). Appropriate life stages of each non-target species were tested with the candidate parasitoid in no choice assays that were paired with comparable stages of the target pest served as positive controls. Following the completion of host-specificity testing, host-specificity hazard indexes for each non-target species tested, as well as for all the non-target species tested together (overall hazard indexes) were calculated and used to categorize the potential risk of introducing *S. galinae* for classical biological control of *A. planipennis*.

Results from our study showed that although some non-target wood-boring insects may be potentially attacked by *S. galinae*, the hazard index (relative to the target host) for those potentially susceptible non-target wood borers is extremely low. This suggests that there is a low risk to non-target species if *S. galinae* were introduced for classical biological control of the invasive *A. planipennis*. Findings of this case study suggest that appropriate positive (target) controls should be included in the experimental design of host specificity testing with candidate biocontrol agents. With the inclusion of such positive controls, host-specificity hazard ratio can be calculated and used to categorize the risks posed by candidate arthropod predators or parasitoids. This approach would allow the quantification of potential risks associated with their introductions for biological control, and greatly facilitate communication of possible risks to regulators and general public.

## References

Belokobylskij, S. A., G. I. Yurchenko, J. S. Strazanac, A. Zaldívar-Riverón, and V. Mastro. 2012. A new emerald ash borer (Coleoptera: Buprestidae) parasitoid species of *Spathius* Nees (Hymenoptera: Braconidae: Doryctinae) From the Russian Far East and South Korea. Ann. Entomol. Soc. Amer. 105: 165–178.

Bigler, F., D. Babendreier, and U. Kuhlmann (eds.) 2006. Biological Control of Arthropods Using Invertebrates: Methods for Environmental Risk Assessment. CABI Publishers, Wallingford, UK.

Burgman, M. 2005. Risks and Decisions for Conservation and Environmental management. Cambridge University Press, Cambridge, UK.

Duan, Jian J., Yurchenko, G. and Fuester, R. W. 2012. Occurrence of emerald ash borer (Coleoptera: Buprestidae) and biotic factors affecting its immature stages in the Russian Far East. Environ. Entomol. 41: 245–254.

Sheppard, A. W., Klinken R. D. V., and T. A. Heard. 2005. Scientic advances in the analysis of direct risks of weed biological control agents to nontarget plants. Biol. Control 35: 215–226.

Van Driesche R. G., and R. Reardon. 2004. Assessing Host Ranges for Parasitoids and Predators Used for Classical Biological Control: A guide to Best Practice. Forest Health Technology Enterprise Team – Morgantown, West Virginia, USA.

Wapshere, A. J. 1974. A strategy for evaluating the safety of organisms for biological weed control. Ann. Appl. Biol. 77: 201–211.

Zwölfer, H. and P. Harris. 1971. Host specificity determination for biological control weeds. Annu. Rev. Entomol. 16: 159 – 178.

Table 1. List of non-target wood-boring insects selected for host specificity testing with *Spathius galinae* for ecological risk assessment for classical biological control of the emerald ash borer (*Agrilus planipennis*).

| **Non-target species** | **Family** | **Order** | **Host Plant** | **Justification for Testing** |
|---|---|---|---|---|
| *Agrilus subcinctus* | Buprestidae | Coleoptera | *Fraxinus* spp. | Congener/same host plants/Native |
| *Agrilus masculinus* | Buprestidae | Coleoptera | *Acer* spp. | Congener, native |
| *Agrilus anxius* | Buprestidae | Coleoptera | *Betula* spp. | Congener, native |
| *Agrilus bilineatus* | Buprestidae | Coleoptera | *Fagus* sp, *Quercus* spp, *Carpinus* spp. *Castinea* spp. | Congener, native |
| *Agrilus auroguttatus* | Buprestidae | Coleoptera | *Quercus spp.* | Congener, native |
| *Chrysobothris* fermorata | Buprestidae | Coleoptera | *Fraxinus* spp., Acer spp. | Same family/host plants, native |
| *Chrysobothris* sexignata | Buprestidae | Coleoptera | Acer spp. | Same family, native |
| *Neoclytus acuminatus* | Cerambycidae | Coleoptera | *Fraxinus* spp., Acer spp. | Same Order/host plants, native |
| *Elaphidion mucronatum* | Cerambycidae | Coleoptera | *Acer* spp. | Same Order, native |
| *Graphisurus fasciata* | Cerambycidae | Coleoptera | *Acer* spp. | Same Order, native |
| *Anoplophora glabripennis* | Cerambycidae | Coleoptera | *Acer* spp., Fraxinus spp., others | Same Order, exotic (same origin) |
| *Hylesinus aculeatus* | Curculionidae | Coleoptera | *Fraxinus* spp. | Same Order/host plants, native |
| *Isorhipis obliqua* | Eucnemidae | Coleoptera | *Betula* spp. | Same Order, native |
| *Xiphydria maculata* | Xiphydriidae | Hymenoptera | Accer spp. | Different Order/host plants, native |
| *Janus abbreviatus* | Cephidae | Hymenoptera | *Salix* spp., *Hibiscus* spp. | Different Order/host plants, native |
| *Podosesia aureocincta* | Sesiidae | Lepidoptera | *Fraxinus* spp. | Different Order/same host plants, native |

# Session 2: The Importance of Considering Intraspecific Variation in Classical Biological Control

## Ecology and genetics of three classical biocontrol introductions to New Zealand

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Most introductions of species to new regions, either intentional or accidental, fail to establish self-sustaining populations (Williamson and Fitter 1996). Even in classical biological control of insects, where natural enemies are intentionally introduced to help suppress insect pests, only ≈30% establish and ≈20% provide some degree of control (Hall et al. 1980). Such low success rates have prompted pleas for greater adoption of experimental approaches to help biological control become a more predictive science (Roderick and Navajas 2003, Marsico et al. 2010).

In New Zealand, classical biological control programs against three accidentally introduced weevils (Coleoptera: Curculionidae), *Listronotus bonariensis* (Kuschel), a South American species, and *Sitona discoideus* Gyllenhal and *S. lepidus* Gyllenhal, both Palearctic, have been used as opportunities to investigate what factors contribute to successful biological control. In particular, the research evaluated the role of parasitoid and host intraspecific genetic variation in the program outcomes. All three programs involved introductions of *Microctonus* spp. parasitoids (Hymenoptera: Braconidae).

The *L. bonariensis* program involved the introduction of two genetically differentiated biotypes of *M. hyperodae* Loan (Hymenoptera: Braconidae) collected from disparate locations in southern South America (Phillips et al. 2008b). One *M. hyperodae* biotype was found in Chile (termed 'western'), and the other in Argentina, Uruguay and Brazil (termed 'eastern'). Both were released together at locations throughout New Zealand, the eastern biotype quickly became dominant nearly everywhere, and this rapid adaptive evolution enhanced the efficacy of the biological control program (Phillips et al. 2008b).

Explaining the greater success of the eastern biotype in New Zealand has proved to be difficult. It cannot be accounted for by climatic matching because the South American source locations of eastern and western *M. hyperodae* share similar climatic matches with New Zealand (Phillips et al., unpublished). In South America, the east-west genetic structure seen in *M. hyperodae* (Iline and Phillips 2004, Winder et al. 2005) does not correspond with the pattern of genetic variation seen in its host, *L. bonariensis* (Lenney Williams et al. 1994), and there is no evidence that the eastern biotype is better adapted to the subset of *L. bonariensis* genotypes found in New Zealand (Phillips et al. 2008b; Phillips et al., unpublished). Other *M. hyperodae* attributes such as egg load (Phillips and Baird 2001), longevity (Goldson et al. 1995) and development rate (Barlow et al. 1994) also showed little variation between the biotypes. However, despite the absence of development rate variation in laboratory experiments, a population dynamics study observed that eastern *M. hyperodae* adults first appear in the field in early summer approximately one month before western adults (Phillips et al., unpublished), and this is the current focus of efforts to explain the competitive advantage of the eastern biotype in New Zealand.

The programs against *S. discoideus* and *S. lepidus* involved introductions of different biotypes of *M. aethiopoides* Loan (Hymenoptera: Braconidae) from various parts of Europe. This parasitoid has also been introduced as a biological agent to North America and Australia. We examined genetic variation in *M. aethiopoides* from much of its natural and introduced ranges to evaluate why introductions to some regions had been successful and others not. The data showed that *M. aethiopoides* genetic variation is almost independent of geographic location, is strongly correlated with host species (Phillips et al. 2008a, Vink et al. 2012), and is perhaps even more closely correlated with the host plant of the host species (Vink et al. 2012). Indeed, most of the genetic variation found in *M. aethiopoides* sampled from 14 countries was also recovered by sampling several host species from various host plants growing in two proximal fields in south eastern France (Phillips et al. 2008a).

Host specialization by *M. aethiopoides* became clearly evident in New Zealand where a biotype reared from *S. discoideus* collected in Morocco successfully controlled *S. discoideus*, a pest of lucerne/ alfalfa (Kean and Barlow 2000), but was unable to complete its development on *S. lepidus*, a pest of white clover (Barratt et al. 1997). In Europe, *S. lepidus* can be a suitable host for *M. aethiopoides* (Aeschlimann 1980), and New Zealand’s population of *S. lepidus* was also found to be susceptible to parasitism by some European *M. aethiopoides* (Phillips et al. 2002). A subsequent introduction of *M. aethiopoides* reared from *S. lepidus* collected in Ireland proved effective against *S. lepidus* in New Zealand (Phillips et al. 2010, Gerard et al. 2011).

Most of the biological control programs that attempted to exploit *M. aethiopoides* in New Zealand, Australia and North America pre-dated the advent of rapid and inexpensive PCR-based genetic analysis methods, and assumed that *M. aethiopoides* genetic variation would vary more between locations than between host species (but see Aeschlimann 1983). Some of these biological control introductions had unsuccessful outcomes, almost certainly because parasitoids collected in the native range were not reared from the same host species as the one being targeted for control in the region of introduction. As with *M. hyperodae*, climatic similarities between source locations and regions of introduction do not appear to be important determinants of success for biological control with *M. aethiopoides*.

**Summary.** *Microctonus* spp. intraspecific genetic variation has proven critical to the outcomes of several biological control programs in New Zealand, and probably also elsewhere. Intraspecific genetic variation in introduced populations increases the probability they will evolve to become locally adapted. Identifying efficacious natural enemy biotypes prior to making a biological control introduction increases the probability of successfully suppressing the targeted pest, and reduces the risk of negative impacts on non-targeted species.

## References

Aeschlimann, J.-P. 1980. The *Sitona* (Col. Curculionidae) species occurring on *Medicago* and their natural enemies in the Mediterannean region. Entomophaga 25: 139-153.

Aeschlimann, J.-P. 1983. Notes on the variability of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae: Euphorinae). Contributions of the American Entomological Institute 20: 329-335.

Barlow, N. D., S. L. Goldson, and M. R. McNeill. 1994. A prospective model for the phenology of *Microctonus hyperodae*, a potential biological control agent of Argentine stem weevil in New Zealand. Biocontrol Sci. Technol. 4: 375-386.

Barratt, B. I. P., A. A. Evans, and C. M. Ferguson. 1997. Potential for biocontrol of *Sitona lepidus* Gyllenhal by *Microctonus spp*. Proceedings of the 50th New Zealand Plant Protection Conference: 37-40.

Gerard, P., D. Wilson, and T. Eden. 2011. Field release, establishment and initial dispersal of Irish Microctonus aethiopoides in *Sitona lepidus* populations in northern New Zealand pastures. BioControl: 1-10.

Goldson, S. L., M. R. McNeill, J. R. Proffitt, and A. A. Hower. 1995. An investigation into the reproductive characteristics of *Microctonus hyperodae* (Hymenoptera: Braconidae), a parasitoid of *Listronotus bonariensis* (Kuschel) (Col.: Curculionidae). Entomophaga 40(3/4): 413-426.

Hall, R. W., L. E. Ehler, and B. Bisabri-Ershadi. 1980. Rate of success in classical biological control of arthropods. Bulletin of the Entomological Society of America 26: 111–114.

Iline, I. I., and C. B. Phillips. 2004. Allozyme markers to help define the South American origins of *Microctonus hyperodae* (Hymenoptera: Braconidae) established in New Zealand for biological control of Argentine stem weevil. B. Entomol. Res. 94: 229-234.

Kean, J. M., and N. D. Barlow. 2000. Long-term assessment of the biological control of *Sitona discoideus* by *Microctonus aethiopoides* and test of a model. Biocontrol Sci. Techn. 10: 215-221.

Lenney Williams, C., S. L. Goldson, D. B. Baird, and D. W. Bullock. 1994. Geographical origin of an introduced insect pest, *Listronotus bonariensis* (Kuschel), determined by RAPD analysis. Heredity 72: 412-419.

Marsico, T. D., J. W. Burt, E. K. Espeland, G. W. Gilchrist, M. A. Jamieson, L. Lindström, G. K. Roderick, S. Swope, M. Szűcs, and N. D. Tsutsui. 2010. PERSPECTIVE: Underutilized resources for studying the evolution of invasive species during their introduction, establishment, and lag phases. Evol. Appl. 3: 203-219.

Phillips, C. B., and D. B. Baird. 2001. Geographic variation in egg load of *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) and its implications for biological control success. Biocontrol Sci. Techn. 11: 371-380.

Phillips, C. B., C. J. Vink, A. Blanchet, and K. A. Hoelmer. 2008a. Hosts are more important than destinations: What genetic variation in *Microctonus aethiopoides* (Hymenoptera: Braconidae) means for foreign exploration for natural enemies. Mol. Phylogenet. Evol. 49: 467-476.

Phillips, C. B., M. R. McNeill, S. Hardwick, C. M. Ferguson, and J. M. Kean. 2010. South Island distributions of clover root weevil and its biocontrol agent New Zealand Grasslands Association 72: 227 - 232.

Phillips, C. B., R. P. Cane, J. Mee, H. M. Chapman, K. A. Hoelmer, and D. Coutinot. 2002. Intraspecific variation in the ability of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) to parasitise *Sitona lepidus* Gyllenhal (Coleoptera: Curculionidae). New Zeal. J. Agr. Res. 45: 295-303.

Phillips, C. B., D. B. Baird, I. I. Iline, M. R. McNeill, J. R. Proffitt, S. L. Goldson, and J. M. Kean. 2008b. East meets West: Adaptive evolution of an insect introduced for biological control. J. Appl. Ecol. 45: 948-956.

Roderick, G. K., and M. Navajas. 2003. Genes in new environments: genetics and evolution in biological control. Nat. Rev. 4: 889-899.

Vink, C. J., B. I. P. Barratt, C. B. Phillips, and D. M. Barton. 2012. Moroccan specimens of *Microctonus aethiopoides* spice our understanding of genetic variation in this internationally important braconid parasitoid of adult weevils. BioControl 57: 751-758.

Williamson, M., and A. Fitter. 1996. The varying success of invaders. Ecology 77: 1661-1666.

Winder, L. M., C. B. Phillips, C. Lenney Williams, R. C. Cane, K. Paterson, C. J. Vink, and S. L. Goldson. 2005. PCR-based DNA analysis corroborates phenotypic evidence of trans-Andean variation in the South American parasitoid, *Microctonus hyperodae*. B. Entomol.l Res. 95: 289-298.

# Classical biological control of the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), using the exotic parasitoid, *Psyttalia lounsburyi* (Hymenoptera: Braconidae) in France : Will the intraspecific hybridization improve the establishment of the biological control agents?

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The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera Tephritidae), is a monophagous insect, developing in cultivated and wild olives (Nardi et al. 2005). Because of its worldwide geographic distribution, it is responsible for economic losses of approximately US$ 800 millions per year (Daane and Johnson 2010, Montiel Bueno and Jones 2002). Despite numerous previous attempts to find efficient natural enemies, biological control of this pest fails. As a consequence, the control of the olive fruit fly still relies on the use of insecticides with consecutive drawbacks for the environment and human health. During the last years, North American researchers (Californian Department of Food and Agriculture & European Biological Control Laboratory) have investigated possible natural enemies, in particular in Africa. The endoparasitoid *Psyttalia lounsburyi* (Silvestri) (Hymenoptera: Braconidae) has been identified as one candidate biological control agent (Daane et al. 2008). This species is found in Kenya, Namibia and South Africa and is considered as a specialist of *Bactrocera* species (Copeland et al. 2004, Mkize et al. 2008, Rugman-Jones et al. 2009).

In the frame of international collaborations, this species has also been evaluated in France with two objectives: 1) to establish perennial populations of *P. lounsburyi*; 2) to investigate to what extent intraspecific hybridization may contribute to improve the success of the biological control agent. The underlying rationale of this second objective comes from the field of invasion biology. Indeed, beneficial hybridizations have been recorded in invasive plants (Ellstrand and Schierenbeck 2000, Lavergne and Molofsky 2007, Rosenthal et al. 2008, Culley and Hardiman 2009, Keller and Taylor 2010) and in some invasive animals (Kolbe et al. 2004; Facon et al. 2005, 2008). The two potential causal mechanisms are heterosis and/or the increase of genetic and phenotypic variance (Rieseberg et al. 1999, Ellstrand and Schierenbeck 2000, Facon et al. 2005).

Within this frame, the molecular characterization of available *P. lounsburyi* populations was carried out using one mitochondrial marker (Cytochrome oxidase c subunit 1) and eight microsatellite markers (Bon et al. 2008, Cheyppe-Buchmann et al. 2011). Two well-supported clusters separating populations from South Africa to those originating from Kenya and Namibia populations were thus distinguished, evidencing a significant differentiation for neutral markers. In parallel, *P. lounsburyi* was found to be highly infected by *Wolbachia*, a bacterial endosymbiont known to potentially induce reproductive barriers between conspecific individuals because of cytoplasmic incompatibility (Stouthamer et al. 1999, Werren 1997). Two *Wolbachia* were more precisely detected, their distribution varying according with the *P. lounsburyi* populations, the sex of the wasps and the rearing generation (Cheyppe-Buchmann et al. 2011, Warot et al. in press, Cheyppe-Buchmann et al. unpublished). All these results helped thus us to select the two most interesting *P. lounsburyi* populations and motivate the removal of their microbial endosymbionts through antibiotherapy.

The two aposymbiotic populations of *P. lounsburyi* (one from Kenya, one from South Africa) as well as their hybrids (F1 and back-crosses) were then compared in laboratory conditions for several phenotypic traits (e.g. potential and realized fecundity) (Benvenuto et al. 2012). Taken as the whole, our results evidenced a significant differentiation between the two parental strains, the South African one exhibiting in general better performances but this trend being modulated for instance by the developmental temperature. The phenotypes of the hybrids were located in-between the phenotypes of their parents. Although heterosis could be thus excluded, these results evidenced that the crossing between these two allopatric populations can generate a high variability for quantitative traits supposed to be involved in the fitness. The underlying genetic determinisms of these traits were nevertheless more or less complex, implying for instance for potential fecundity both additivity and cytonuclear interactions.

Given the results obtained in laboratory conditions, the deliberate introduction of the two *P. lounsburyi* strains in the field was a rare opportunity to document the actual importance of genetic differences on the establishment's success as well as on the post-release evolution. A first session of releases was thus organized in 2008. A total of about 42000 individuals were thus mass-reared and distributed in 60 sites according to 3 modalities (only Kenyan individuals, only South African individuals or a mix of both populations / site). Post-release surveys evidenced that *P. lounsburyi* was able to find its hosts and successfully develop in some locations but no more individuals were recovered after the second winter (Malausa et al. 2010, Borowiec et al. 2012). One possible explanation of this failure was the quite low propagule pressure per site (about 700 individuals). A second session was thus organized in 2012 using the same amount of biological control agents but only two locations and one modality (mix of both parental populations. Even if some offspring were recovered some weeks after the releases, further investigations will be necessary to assess the fate of these new introductions.

## References

Benvenuto, C., S. Cheyppe-Buchmann, G. Bermond, N. Ris, and X. Fauvergue. 2012. Intraspecific hybridization, life history strategies and potential invasion success in a parasitoid wasp. Evol. Ecol. 26: 1311-1329.

Bon, M. C., W. Jones, C. Hurard, A. Loiseau, N. Ris, C. Pickett, A. Estoup, and X. Fauvergue. 2008. Identification of 21 polymorphic microsatellites in the African parasitoid wasp, *Psyttalia lounsburyi* (Silvestri) (Hymenoptera : Braconidae). Mol. Ecol. Resour. 8: 930–932.

Borowiec, N., G. Groussier-Bout, E. Vercken, M. Thaon, A. Auguste-Maros, S. Warot, G. Delvare, N. Ris, X. Fauvergue, and J. C. Malausa. 2012. Diversity and geographic distribution of the indigenous and exotic parasitoids of the olive fruit fly, *Bactrocera oleae* (Diptera : Tephritidae), in Southern France. IOBC-WPRS Bulletin 79: 71-78.

Cheyppe-Buchmann, S., M. C. Bon, S. Warot, W. Jones, T. Malausa, X. Fauvergue, and N. Ris. 2011. Molecular characterization of *Psytallia lounsburyi*, a candidate biocontrol agent of the olive fruit fly, and its *Wolbachia* symbiont as a pre-requisite for future intraspecific hybridization. Biocontrol 56: 713-724.

Copeland, R. S., I. M. White, I. Okumu, P. Machera, and R. A. Wharton. 2004. Insects associated with fruits of Oleaceae (Asteridae, Lamiales) in Kenya, with special references to Tephritidae (Diptera). Bishop Museum Bulletin of Entomology 12: 135–164.

Culley, T. M., and N. A. Hardiman. 2009. The role of intraspecific hybridization in the evolution of invasiveness: a case study of the ornamental pear tree *Pyrus calleryana*. Biol. Invasions 11: 1107–1119.

Daane, K. M., and M. W. Johnson. 2010. Olive fruit fly: managing an ancient pest in modern times. Annual Review of Entomology 55: 151–169.

Daane, K. M., K. R. Sime, X. Wang, H. Nadei, M. W. Johnson, M. W. Walton, A. Kirk, and C.H. Pickett. 2008. *Psyttalia lounsburyi* (Hymenoptera: Braconidae), potential biological control agent for the olive fruit fly in California. Biol. Control 44: 78–89.

Ellstrand, N. C., and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? P. N. Acad. Sci. USA 97: 7043–7050.

Facon, B., P. Jarne, J. P. Pointier,and P. David. 2005. Hybridization and invasiveness in the freshwater snail *Melanoides tuberculata*: hybrid vigour is more important than increase in genetic variance. J. Evol. Biol. 18: 524–535.

Facon, B., J. P. Pointier, P. Jarne, V. Sarda, and P. David. 2008. High genetic variance in life-history strategies within invasive populations by way of multiple introductions. Curr. Biol. 18: 363–367.

Keller, S. R., and D. R. Taylor. 2010. Genomic admixture increases fitness during a biological invasion. J. Evol. Biol. 23: 1720–1731.

Kolbe, J. J., R. E. Glor, L. Rodrıguez Schettino, A. C. Lara, A. Larson, and J. B. Losos. 2004. Genetic variation increases during biological invasion by a Cuban lizard. Nature 431: 177–181.

Lavergne, S., and J. Molofsky. 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. P. N. Acad. Sci. USA 104: 3883–3888.

Malausa, J. C., A. Auguste-Maros, S. Cheyppe-Buchmann, G. Groussier-Bout, N. Ris, M. Thaon, S. Warot, and X. Fauvergue. 2010. IOBC-WPRS Bulletin 59:163-170.

Mkize, N., K. A. Hoelmer, and M. H. Villet. 2008. A survey of fruitfeeding insects and their parasitoids occurring on wild olives, *Olea europaea* ssp *cuspidata*, in the Eastern Cape of South Africa. Biocontrol Sci. Techn. 18:991–1004.

Montiel Bueno, A., and O. Jones. 2002. Alternative methods for controlling the olive fly, *Bactrocera oleae*, involving semiochemicals. IOBC/WPRS Bulletin 25:1–11.

Nardi, F., A. Carapelli, R. Dallai, G.K. Roderick, and F. Frati. 2005. Population structure and colonization history of the olive fly, *Bactrocera oleae* (Diptera, Tephritidae). Mol. Ecol. 14:2729–2738.

Rieseberg, L. H., M. A. Archer, and R. K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity 83: 363–372.

Rosenthal, D. M., A. P. Ramakrishnan, and M. B. Cruzan. 2008. Evidence for multiple sources of invasion and intraspecific hybridization in *Brachypodium sylvaticum* (Hudson) Beauv. in North America. Mol. Ecol. 17: 4657–4669.

Rugman-Jones, P. F., R. Wharton, T. van Noort, and R. Stouthamer. 2009. Molecular differentiation of the *Psyttalia concolor* (Szepligeti) species complex (Hymenoptera: Braconidae) associated with olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), in Africa. Biol. Control 49: 17–26.

Stouthamer, R., J. A. J. Breeuwer, and G. D. D. Hurst. 1999. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. Annu. Rev. Microbiol. 53: 71–102.

Warot, S., M. C. Bon, and N. Ris. 2013. Caractérisation des bactéries endosymbiotiques *Wolbachia* d'un auxiliaire de lutte biologique contre la mouche de l'olive, *Psyttalia lounsburyi.* Cah Tech INRA. in press

Werren, J. H. 1997. Biology of *Wolbachia*. Annu. Rev. Entomol. 42:587–609.

# Variability and evolution of the venom of two biological control agents from the genus *Psyttalia*

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Parasitoid wasps are widely used in biological control programs since they are naturally efficient to control the population of their host because of their antagonistic interactions. These antagonistic interactions lead to strong co-evolution between parasitoid virulence and host resistance. For instance, insect immune response to the intrusion of a foreign object, such as a parasitoid egg, is the encapsulation process, which requires both cellular and humoral components (Carton et al. 2008). To evade or counteract the host immune reaction, endoparasitoids have evolved different strategies (Poirié et al. 2009). The most often described is the injection of venom and/or ovarian factors that can include proteins, vesicular, or virus-like, components (also described as Virus-Like Particles, VLPs) or symbiotic viruses (such as polydnaviruses, PDVs) (Beckage and Drezen, 2012). As a consequence, the success of parasitoids used in biological control is likely to depend on the content of their venom and its efficiency to counteract the host immune reaction.

Previous studies focusing on venom protein content and diversity at inter-specific level have shown that venom is very diversified even between closely-related species (Crawford et al. 2008; Dominique et al. 2012). The intra-specific and intra-population variability can be studied thanks to protein electrophoresis coupled with new methods of image analysis that enable to quantify the variability of individual venom profiles. These methods have recently lead to results showing that the variability is surprisingly high even between individuals (Colinet et al. 2012). This high variability suggests that the evolutionary potential of venom is high and that venom components thus likely vary among populations of candidate biological control agents. This means that the screening of venom might be relevant to monitor biological control agents. Indeed, a high evolutionary potential is expected to affect chances of success of biological control as it is often assumed that establishment relies on adaptation to new environments. However, the high evolutionary potential may also select laboratory-fit individuals. For instance, biological control agents may become more adapted to their alternative host used in an industrial settings than to their natural (and target) host. The screening of such venomic diversity might thus permit following adaptation in response to the laboratory or to the release.

However, such screening would prove useful only if variation of venomic content is correlated with variation of parasitism success against target pests or substitution host used for laboratory rearing. Many venom proteins have already been shown to be involved in parasitism success (Asgari et al. 2003, Colinet et al. 2009). At the intra-specific level, several studies have shown an correlation between variability in host preference and variability in virulence (Kraaijeveld et al. 1995). Moreover, a correlation could be found between quantitative variation of one venom protein essential for parasitism success and variability in virulence (Colinet et al. 2010, Dubuffet et al. 2009).

In this work, we have characterized and compared the venom content of various populations of the biological control agent *Psyttalia lounsburyi* (Silvestri) (Hymenoptera: Braconidae), used against the olive fruit fly *Bactrocera oleae* (Rossi) (Diptera:

Tephritidae). Then, we have studied the evolution of the venom and of the parasitic success of various populations in controlled conditions.

The venom content was characterized by a combined transcriptomic and proteomic approach in order to identify the putative function of venom's proteins and explain the observed variation in venom contents. The venomic variability between individuals was studied at the individual level by 1D SDS-PAGE (Colinet et al. 2012) notably, to compare intra-population and inter population variability.

For this study, we used two *P. lounsburyi* strains from South Africa and Kenya. Both are reared in the laboratory on the substitution host *Ceratitis capitata* (Weideman) (Diptera: Tephritidae) for more than 6 years (i.e. more than 100 generations). Our results reveal quantitative differences for some bands on venom profiles between Kenyan and South African laboratory strains for which original populations are strongly genetically differentiated.

To study the effect of the host shift between the natural and substitution hosts, we have recently obtained new samples from the field in South Africa and Kenya. This allowed us to follow the evolutionary response to the host shift from *B. oleae* to *C. capitata* in several replicates and to compare laboratory strains to wild strains. Preliminary results show a higher inter-individual variability in field populations than in laboratory strains (Fig 1).

Overall, this work provides encouraging results about the relevance of rearing quality-control methods based on venom analysis. Indeed, venom protein electrophoresis is cheap and simple to carry out once venom apparatus are obtained, and they reveal venomic inter-individual variability, which complements the usual quality-control procedures based on phenotypic trait.

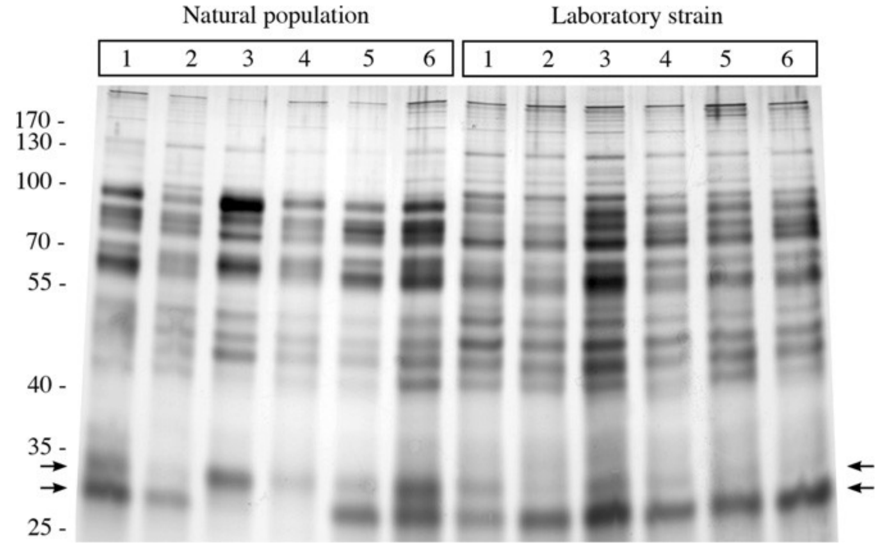

Figure 1. (Reprinted from Colinet et al. 2012)

Electrophoretic comparison of venom proteins from *Psyttalia lounsburyi* individuals. Venom glands were dissected in insect Ringer solution supplemented with a protease inhibitors cocktail (PI; Roche) and residual tissues were removed by centrifugation. The individual protein contents of 12 individual venom glands, was analyzed under reducing conditions on a 12.5% SDS– PAGE and visualized by silver staining. 6 individuals originate from a population sampled in Sirimon Forest (Kenya), the other 6 being issued from a strain collected in Kenya but reared in laboratory conditions for more than 8 years. Arrows point to examples of inter-individual variation with the presence/absence of bands in the natural population or quantitative variation of specific bands in the laboratory strain. Molecular weight standards are in kDa.

## References

Asgari, S., Zhang, G., Zareie, R., and Schmidt, O. 2003. A serine proteinase homolog venom protein from an endoparasitoid wasp inhibits melanization of the host hemolymph. Insect Biochem. Molec. 33: 1017–1024.

Beckage, N., and Drezen, J. 2012. Parasitoid viruses: symbionts and pathogens. 1st ed.

Carton, Yves, Poirié, Marylène, and Nappi, A. J. 2008. Insect immune resistance to parasitoids. Insect Sci. 15: 67–87.

Colinet, D, Mathé-Hubert, H., Allemand, R., Gatti, J. L., and Poirié, M. 2012. Variability of venom components in immune suppressive parasitoid wasps: from a phylogenetic to a population approach. J. Insect Physiol. In press.

Colinet, D., A. Dubuffet, D. Cazes, S. Moreau, J.-M. Drezen, and M. Poirié, 2009. A serpin from the parasitoid wasp *Leptopilina boulardi* targets the Drosophila phenoloxidase cascade. Dev. Comp. Immunol. 33: 681–689.

Colinet, D., A. Schmitz, D. Cazes, and J. Gatti. 2010. The Origin of Intraspecific Variation of Virulence in an Eukaryotic Immune Suppressive Parasite. PLoS Pathogens. 6: 1–11.

Crawford, A.M., R. Brauning, G. Smolenski, C. Ferguson, D. Barton, T.T. Wheeler, and A. McCulloch. 2008. The constituents of Microctonus sp. parasitoid venoms. Insect Mol. Biol. 17: 313-324.

Dubuffet, A., D. Colinet, C. Anselme, S. Dupas, Y. Carton, and M. Poirié, 2009. Variation of *Leptopilina boulardi* success in Drosophila hosts: what is inside the black box? Adv. Parasit. 70: 147–188.

Kraaijeveld, A., B. Nowee, and R. Najem, 1995. Adaptive variation in host-selection behaviour of *Asobara tabida*, a parasitoid of Drosophila larvae. Funct. Ecol. 9: 113–118.

Poirié, M., Y. Carton, and A. Dubuffet. 2009. Virulence strategies in parasitoid Hymenoptera as an example of adaptive diversity. CR. Biol. 332: 311–320.

# How generalist are generalists?

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Parasitoids are frequently used in biological control because of the commonly held belief that they are relatively host specific (Godfray 1994). Not only does this mean that released parasitoids will be most efficient at attacking the target pest species, but it also reduces the possibility of environmental harm through spillover of rapidly-growing parasitoid populations from crops into adjacent natural habitats, as is observed for generalist predators (Rand, 2006). However, parasitoids are seldom specific to the point of attacking only one host species, and there are many parasitoid species that have a great range of hosts (Mackauer and Starý 1967). Parasitoid host range may not be consistent across an entire species, as different races can be specific to different host species (Stireman et al. 2006, Antolin et al. 2006, Abrahamson and Blair 2008, Henry et al. 2008). Thus, the ability of a parasitoid to control different hosts on different host plants may not be constant, even among different genotypes of a single species. Indeed, within the range of all potential hosts, not all of them are equally preferred and/or become susceptible to the development of parasitoids (Desneux et al. 2009). The effectiveness for biological control of a given genotype of a non-specialist parasitoid will depend on its potential "generalism" (i.e. its ability to oviposit and develop on different hosts) and on the balance between its preferences for the target pest versus alternative hosts (including non-pest species). If a certain genotype or race of a parasitoid is too specialized and cannot use alternative hosts when the target pest is unavailable, they may be slow to colonize the crop when the pest appears.

We examine the case of *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) in Chile and the great number of aphid species that it supposedly exploits in different plant hosts and habitats. Field samplings revealed that *A. ervi* is the most common parasitoid species parasitizing the *Acrythosiphum pisum* (Harris) (Hemiptera: Aphididae) complex, representing more than 94% of all parasitoid individuals emerging from this host in two different regions sampled. Additionally, the diversity of parasitoid species associated with *A. pisum* was low, and included parasitoids from the genus *Praon* (*P. volucre* (Haliday) and *P. gallicum* Stáry) and *Aphidius* (*A. matriariacae* Haliday and *A. colemani* Viereck) (Hymenoptera: Braconidae). These parasitoids prone to be highly-habitat (associated to different plant families) and host-aphid generalists (Starý et al. 1994). On cereal aphids, a larger parasitoid diversity was observed in comparison to that observed on the *A. pisum* complex. The aphid *Sitobion avenae* (Fabricius) (Hemiptera: Aphdididae) hosted up to nine parasitoid species, the genus *Aphidius* being the more represented, including *A. ervi* (38%), *A. uzbekistanicus* Luzhetzki (28%), *A. rhopalosiphi* de Stephani-Perez (12%), *A. picipes* (Nees) (3%), *A. colemani* (3%), *A. matricariae* (0.4%), but *P. volucre* (9%), *P. gallicum* (6%), and *Lysiphlebus testaceipes* Cresson (0.5%) were also detected. Furthermore, *S. avenae* and *Rhopalosiphum padi* appeared as the common resource for the same parasitoid assemblage. *Rhopalosiphum padi* (Hemiptera: Aphdidae) was parasitized mostly by *A. colemani* (34%), *A. uzbekistanicus* (22%), *A. rhopalosiphi* (20%) and by *A. ervi* to a lesser extent (11%), but also by *L. testaceipes* (10%), *A. picipes* (2.4%), *P. volucre* (0.4%), *P. gallicum* (0.4%) and *A. matricariae* (0.4%). These observations are in agreement with that previously reported by Starý et al. (1994), Gerding et al. (1989) and Gerding and Figueroa (1989), who described *A. ervi* as the most predominant parasitoid species controlling *S. avenae* in Chile.

From these field results we focused on the virulence and infectivity of four different parasitoid lineages originating from two *A. pisum* races (from *A. pisum* collected from alfalfa and from peas), *S. avenae and R. padi.* The results obtained indicate that natural populations of *A. ervi* coming from different hosts in the field, exhibit important differences in infectivity on their natal host in comparison with non-natal hosts, although this pattern was not observed in all parasitoid populations studied or in the virulence expressed by these populations on the tested hosts. Parasitoids from the aphid *S. avenae* and *A. pisum*-alfalfa race showed a high infectivity on their natal host in comparison to on the assayed non-natal hosts, for some of the behavioral variables studied. This suggests that both parasitoid populations would represent specialist populations in terms of their infectivity to their natal hosts (i.e. preference to hosts) (van Tienderen 1991). On the other hand, *A. ervi* parasitoids from the *A. pisum*-pea race showed a similar infectivity irrespective of the host race assayed, suggesting that this population exhibits a greater phenotypic plasticity for behavioral traits associated with host use, a pattern observable for a generalist population in terms of their infectivity to hosts (van Tienderen 1991). In terms of virulence on three assayed lineages (both *A. pisum* races and *S. avenae*), these showed a similarly high virulence on natal and non-natal hosts, thus evidencing the absence of local adaptation associated to hosts.

Regarding the use of alternative hosts, different evolutionary trajectories could be followed by introduced biological control agents in a new area, the destiny of populations being strongly influenced by human activities, especially for agents that are mass-reared for the release in agriculture landscapes. In this sense, while locally adapted natural enemies should have higher rates of population increase or be more damaging to their local hosts (Hufbauer and Roderick 2005); adaptive phenotypic plasticity may be favored over adaptive differentiation in organisms living in fluctuating environments (Thibert-Plante and Henry 2010). Our results highlight the role of phenotypic plasticity allowing parasitoids to maximize fitness on alternative hosts and thus enabling them to use different aphid species as hosts, which is evidenced by a high gene flow among parasitoid populations associated to different hosts (Zepeda-Paulo et al. unpublished). However, *A. ervi* does not use equally its potential host range, showing significant differences in preference and virulence across hosts possibly suggesting an effect of host phylogeny on those traits. In terms of pest suppressiveness, landscapes with a highly plastic *A. ervi* should be more suppressive than landscapes without them, as they would be more resilient to changes, increasing any insurance effects when facing environmental change. Variations in aphid abundances on alfalfa and gramineous crops should particularly affect specialized parasitoids, reducing resilience of such landscapes. Moreover, introduced biological control agents with the capacity to use and maintain a high performance on alternative hosts could be favored under the current climate change scenario, which predicts a greater frequency and intensity of pest outbreaks due to disruption of parasitoid-herbivore dynamics (Stireman et al. 2005). Indeed, genotype-genotype association has been shown to be altered by a recent increase of local temperatures in an aphid-parasitoid association of a cultivated crop (Lavandero and Tylianakis 2013). All this poses the need to incorporate new eco-evolutionary approaches in the selection process of biological control agents. In addition, this study reaffirms the usefulness of this type experimental approach to detect patterns of adaptation of biological control agents to target hosts; making a clear distinction between infectivity (preference) and virulence of parasitoids, because often both are camouflaged in the measures of adaptation or explicitly focused on infectivity as a measure of host-adaptation (Hufbauer and Roderick 2005). Future research should investigate the effect of high plastic parasitoids on the efficiency of pest control; quantifying the relative frequency and dynamics of these *A. ervi* populations in the field. The role of endosymbionts should not be neglected either, as most likely they have an important part in the abundance and efficacy of biological control agents in the field and their prevalence.

## Acknowledgments

We thank Dr. Jean-Christophe Simon and Dr. Yannick Outreman for comments in the design of the experiments. We also thank Aura Barria, Romina Zepeda and Marcos Dominguez for their assistance in laboratory and field work. This work was funded by FONDECYT 1110341.

## References

Abrahamson, W. G., and C. P. Blair. 2008. Sequential radiation through host-race formation: herbivore diversity leads to diversity in natural enemies. pp, 188-202 In Tilmon, K. J. ed. Specialization, Speciation and Radiation: The Evolutionary Biology of Herbivorous Insects. University of California Press, Berkeley, California.

Antolin, M. F., T. A. Bjorksten, and T. Y. T. Vaughn. 2006. Host-related fitness trade-off in a presumed generalist parasitoid, *Diaeretiella rapae* (Hymenoptera:Aphidiidae). Ecol. Entomol. 31: 242-254.

Desneux, N., E. R. J. Barta, E. K. A. Hoelmer, E. K. R. Hopper, and E. G. E. Heimpel. 2009. Multifaceted determinants of host specificity in an aphid parasitoid. Oecologia 160: 387–398.

Gerding, M.P., E. S. Zuñiga, C. E. Quiroz, H. M. Norambuena, and R. M. Vargas. 1989. Abundancia relativa de los parasitoides de *Sitobion avenae* (F) y *Metopolophium dirhodum* (WLK) (Homoptera:Aphididae) en diferentes áreas geográficas de Chile. Agricultura técnica (Chile) 49: 104-114.

Gerding, M. P. and A. E. Figueroa. 1989. Progeny reduction of *Sitobion avenae* Fab (Homoptera: Aphididae) by Aphidius ervi (Hymenoptera: Aphidiidae). Agricultura tecnica (Chile), 49(1), 50-53.

Godfray, H. C. J. 1994. Parasitoids: behavioral and evolutionary ecology. Princeton, NJ: Princeton University Press, Princeton, NJ.

Henry, L. M., B. D Roitberg, and D. R. Gillespie. 2008. Host-range evolution in *Aphidius* parasitoids: fidelity, virulence and fitness trade-offs on an ancestral host. Evolution 62: 689–699.

Hufbauer, R. A. and G. K. Roderick. 2005. Microevolution in biological control: mechanisms, patterns, and processes. Biol. Control 35: 227–239.

Lavandero, B. and J. Tyliankis. 2013. Genotype matching in a parasitoid-host genotypic food web: an approach for measuring effects of environmental change. Mol. Ecol. 22: 229-238.

Mackauer, M. and P. Starý. 1967. Hymenoptera. Ichneumonoidea. World Aphidiidae. In Index of Entomophagous Insects (V. Delluchi and G. Remaudière eds.). Le Francois, Paris, pp. 1–195.

Rand, T. A., J. M. Tylianakis and T. Tscharntke. 2006. Spillover edge effects: the dispersal of agriculturally subsidized insect natural enemies into adjacent natural habitats. Ecol. Lett. 9:603–614.

Starý, P., M. Gerding, H. Norambuena and G. Remaudière. 1994. Environmental research on aphid parasitoid biocontrol agents in Chile (Hym, Aphidiidae, Hom, Aphidoidea). J. Appl. Entomol. 115: 292–306.

Stireman, J. O.III., J. D. Nason and S. B. Heard. 2005. Host-associated genetic differentiation in phytophagous insects, general phenomenon or isolated exceptions? Evidence from a goldenrod insect community. Evolution 59:2573–2587.

Stireman, J. O.III., J. D. Nason and S. B. Heard, and J. M. Seehawer, 2006. Cascading host-associated genetic differentiation in parasitoids of phytophagous insects. P. R. Soc. B 273: 523–530.

Thibert-Plante, X. and A. P. Hendry. 2010. The consequences of phenotypic plasticity for ecological Speciation. J. Evol. Biol. 24: 326–342.

van Tienderen, P. H. 1991. Evolution of generalists and specialists in spatially heterogeneous environments. Evolution 45: 1317-1331.

# Biological control of mealybugs (Hemiptera, Pseudococcidae) in apple orchards and ornamental plants in France

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Mealybugs are an increasingly important problem in French orchards, greenhouses and ornamental plants, probably as a consequence of the decreasing use of broad-spectrum pesticides.

In the early 2000’s, a biological control program was launched against the mealybug *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococidae) in orchards (apple) and greenhouses (tomato, strawberry). Evaluations of the parasitoids *Acerophagus flavidulus* (Bréthes), and *A. maculipennis* (Mercet) (Hymenoptera: Encrytidae and the commercially available coccinellid *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) have been conducted until 2008, and resulted in releases of a French mass-reared population of *A. flavidulus*. Although no accurate post-release monitoring could be performed, contrasted levels of control success were observed soon after the releases and sample collections after two years evidenced the successful establishment of the natural enemy in several sites.

During the biological control program against *P. viburni*, another mealybug species, *Pseudococcus comstocki* (Kuwana) (Hemiptera: Pseudococcidae) was detected in 2005, mainly on apple and pear orchards (often in sympatry with *P. viburni*), as well as *Morus* spp. (*Moraceae*) ornamental trees. A biological control program was started against *P. comstocki* in 2009. Parasitoids known as specific to *P. comstocki* were found on *P. comstocki* populations genetically similar to that of France: *Acerophagus malinus* (Gahan), *Clausenia purpurea* Ishii (Hymenoptera: Encyrtidae), *Allotropa burrelli* Musebeck (Hymenoptera: Platygastridae). Unexpectedly, *Acerophagus malinus* was also found on ornamental trees in Southeastern France. The three parasitoid species were evaluated in the laboratory and two (*A. burrelli* and *A. malinus)* are currently mass-reared. First releases of *A. malinus* have been performed in 2012, while releases of both species are planned in orchards and ornamental trees in 2013- 2014.

In these biological control programs, we have tried to investigate whether or not taking into account intraspecific variation can impact the efficiency of the programs, at any of its steps. Intraspecific variation is likely important for at least three reasons (Hufbauer and Roderick 2005; Phillips et al. 2008). First, natural enemy or pest populations from various geographical regions may have diverged genetically and/or ecologically over time. Hence, not all populations of natural enemies may be efficient against the target pest population in a specific geographical region. It is even likely that some pests are actually complexes of cryptic species, which may respond differently to a same natural enemy species. Second, more generally, natural enemy populations likely vary in terms of overall performance as biological control agents for a set of reasons (differential tolerance to environmental conditions, fixed deleterious alleles in the genomes displayed by all individuals of a population, etc.). Third, some populations likely carry more genetic diversity, which may be advantageous for adaptation to new environmental conditions (e.g. occurring during the establishment to laboratory or field conditions).

In the *P. viburni* program, we have studied 1) whether *A. flavidulus* populations from different countries (France, Chile) performed differently on French populations of *P. viburni*, and 2) whether the parasitoid performs differently when attacking mealybugs originating from different host plants (tomato, apple tree). The results showed that the host plant had more effect that the geographical origin of the parasitoid populations.

After the failure of biological control releases at some sites of North-Western France, we also genotyped microsatellite markers of French populations of *P. viburni* to check if the failures could be due to the occurrence of differentiated populations. The situation was not completely clarified by the genetic analysis: although the populations where biological failed were highly genetically differentiated to other populations, it was not clear whether these populations had simply suffered a severe genetic bottleneck or experienced a very divergent evolutionary history.

In the *P. comstocki* program, research on candidate biological control agents was assisted by DNA characterization, using the markers described in Malausa et al. (2011). We first characterized the mealybugs pre-identified by rapid morphological examination as *P. comstocki*, from Italy, Syria, China, Japan and Turkey. Then, we characterized the parasitoids infesting these populations. Molecular identifications of the mealybugs revealed that populations from Syria were actually a closely related species, *Pseudococcus cryptus* Hempel (Hemiptera: Pseudococcidae), and that those from China were very different to the French *P. comstocki* and might also be another species. Here, the DNA identification probably avoided taxonomic confusion that may have impacted the biological control program. In addition, the identification of parasitoids allowed the detection of variation within each species collected from the various countries, and guided our choice of parasitoid importation.

Then, we compared in the laboratory the performance of several populations of a same species, differing by their geographical origin and displaying DNA differences within mitochondrial and nuclear sequences). This work was performed for both *A. malinus* and *A. burrelli*, the two species imported in the laboratory. We studied the variability several phenotypic traits (fecundity, sex-ratio, longevity, emergence rate, etc.) over two generations. When looking at the first generation only, significant differences between populations were observed, but the results obtained at the second generation proved highly contradictory, showing that most variation was rather caused by micro-environmental variation in the laboratory. However, comparisons among populations and inbred lines of *A. burrelli* revealed variation of generation time (related to pupation duration).

Finally, we investigated whether the parasitoid populations imported in the laboratory have undergone loss of genetic diversity since their establishment in the lab, despite the high densities at which they were maintained (>30 individuals at the first generations in the laboratory, and > 500 individuals at later generations). Genotyping of microsatellite markers revealed a small loss of allelic richness, no significant loss of average heterozygosity and revealed that only rare alleles were probably lost over the rearing process. Our team is currently performing experiments to test whether higher genetic diversity influences positively population establishment and performance in laboratory and field conditions.

## References

Hufbauer R. A., and G.K. Roderick. 2005. Microevolution in biological control: Mechanisms, patterns, and processes. Biol. Control 35:2 27–239.

Malausa, T., A. Fenis, S. Warot, J.-F. Germain, N. Ris, E. Prado, M. Botton, F. Vanlerberghe-Masutti, R. Sforza, C. Cruaud, A. Couloux, and P. Kreiter. 2011. DNA markers to disentangle complexes of cryptic taxa in mealybugs (Hemiptera: Pseudococcidae). J. Appl. Entomol. 135: 142–155.

Phillips C. B., D. B .Baird, I. I. Iline, M. R. McNeill, J. R. Proffitt, S. L. Goldson, and J. M. Kean. 2008. East meets West: Adaptive evolution of an insect introduced for biological control. J. Appl. Ecol. 5: 948–956.

# Survey and efficacy of the Western European *Anagyrus* near *pseudococci* (Hymenoptera: Encyrtidae) on *Planococcus ficus* (Hemiptera: Pseudococcidae), a pest in California vineyards

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## Introduction

The vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) was first reported in California vineyards in 1994. It negatively impacts wine and table grapes by directly decreasing both the vigor and quality. In addition, the species is known to efficiently transmit ampeloviruses inducing leafroll disease in many grape cultivars (Daane et al. 2012). Control by chemicals has been ineffective so biological control was investigated in depth using the parasitoid encyrtid wasp, *Anagyrus* near *pseudococci* (Girault) (Hymenoptera: Encyrtidae).

Although *A. pseudococci* (Girault) (Hymenoptera: Encyrtidae) seems to be the best potential agent to control populations of *P. ficus*, it is not as effective as expected, as populations of *P. ficus* continue to threaten California. This failure can be explained by the genetic mismatch between the biological control agent and its host. Recent studies of *P. ficus* and *A. pseudococci* have demonstrated significant genetic variations between isolated populations in the native range. Triapitsyn et al. (2007) showed that the species known as *A. pseudococci* was divided morphologically into several species. These results suggest that biology and/or physiology of *A. pseudococci* and *A.* near *pseudococci* (new taxon) may differ significantly. These differences could then have an impact on the efficiency of the parasitism of *P. ficus*. In addition, although mealybugs are morphologically very similar, a recent study on Pseudococcidae highlighted interspecific and intraspecific differences, as shown with *Planococcus* spp. (Malausa et al. 2010). These genetic differences among populations of *A. pseudococci* and *P. ficus* suggest that interactions in this host/parasitoid system are complex and subtle. By exploiting the genetic differences observed between various strains of *P. ficus* and *A. pseudococci*, we hope to increase knowledge of this system to improve the efficiency of the biological control program in California. The aim of our study was to compare the impact of two populations of *A. pseudococci,* an Israeli strain that has already been released in California, and a French strain, not yet released against the Californian *P. ficus*. Various biological parameters were measured under laboratory conditions including: (1) parasitism rate as a measure of the efficiency of each strain; (2) sex-ratio and female development time as indicators of the potential number of generations per year, and (3) the size of emerged females.

## Material & Methods

*Field survey:* Surveys were conducted throughout the Mediteranean basin. Three populations of *Planococcus ficus* (*Pf*) were obtained from California (Berkeley University), Israel (Bet Dagan), and France (field-collected at Limoux, Aude). The parasitoid *A. pseudococci* was field-collected near Limoux (Aude) in Southern France, and a commercially produced strain of the Israeli strain was obtained. Both insects were reared out in a quarantine laboratory environmental chamber on organic sprouting potatoes (Temperature: 24°C; photoperiod: 16h:8h; RH: 80%).

*Laboratory experiments:* All combinations of tests were conducted in the quarantine laboratory with inexperienced females of *A. pseudococci* placed in contact with a male for 24 hrs. For each test, at hrs 0 and 10, 3rd larval stage/adult mealybugs were placed in a square plastic box with a 2 cm-long sprout of potato. Inexperienced *Ap* females (French or Israeli) were placed with L3/adult mealybugs of either the Californian, French, or Israeli strain of *P. ficus*. A total of four combinations "parasitoid strain/mealybug strain" was tested (Figure 1).

## Results & Discussion

Our data suggest that the Israeli strain of *A. pseudococci* has a greater impact on the Californian *P. ficus* than the French strain. Indeed, rate of puncture, rate of acceptance and effective parasitism rate were significantly higher for the Israeli strain at 90.3%, 99.6% and 16% respectively, than the French 69.7%, 85.3% and 9.8%, respectively. We do not recommend the French *A. pseudococci* for future releases into California. Interactions between immune defenses of *P. ficus* and their avoidance by *A. pseudococci* will also be discussed. Additional tests with populations of *A. pseudococci* of different geographical origins are needed to improve the efficiency of the biological control of *P. ficus* in California vineyards. We will discuss the efficacy of the Italian and Spanish strains as well in regard of the phylogeography of *P. ficus*.

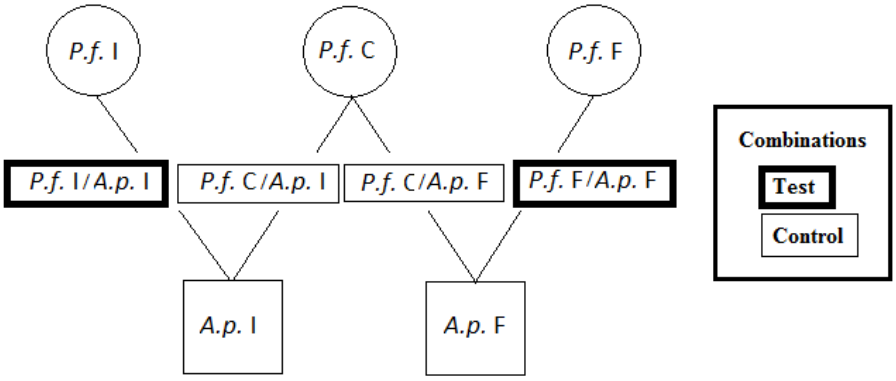
## References

Daane, K. M., R. P. P. Almeida, , V. A. Bell, M. Botton, M. Fallahzadeh, M. Mani, J. L. Miano, R. Sforza, V. M. Walton, and T. Zaveizo. 2012. Biology and management of mealybugs in vineyards. p. 271-307. In: N.J. Bostanian, C. Vincent, R. Isaacs (eds.), Arthropod Management in Vineyards: Pests, Approaches and Future Directions. Springer, Dordrecht, NL.

Malausa M., A. Fenis, S. Warot, J. F. Germain, N. Ris, E. Prado, M. Botton, F. Vanlerberghe-Masutti, R. Sforza, C. Cruaud, A. Couloux, and P. Kreiter. 2010. DNA markers to disentangle complexes of cryptic taxa in mealybugs (Hemiptera: Pseudococcidae). J. Appl. Entomol. 135:142-155.

Triapitsyn, S. V., D. González, D. B. Vickerman, J. S. Noyes, and E. B. White. 2007. Morphological, biological, and molecular comparisons among the different geographical populations of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae), parasitoids of *Planococcus* spp. (Hemiptera: Pseudococcidae), with notes on *Anagyrus dactylopii*. Biol. Control. 41**,** 14-24.

**Figure 1: Diagram representing combinations of mealybug population / parasitoid population used for the experiments between *Anagyrus pseudococci* (Hym., Encyrtidae) females and *Planococcus ficus* (Hem., Pseudococcidae) (*P.f.* = *Planococcus ficus*; *A.p.* = *Anagyrus pseudococci*; C = California; F = France; I= Israel).**



# Session 3: Recent Classical Biological Control Projects Against Forest Pests

## Biology and establishment of *Selitrichodes neseri*, a new biological control agent for the eucalyptus gall wasp, *Leptocybe invasa*

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Insect pests pose a serious threat to sustainable *Eucalyptus* plantation forestry worldwide. This includes native insect pests, mostly generalists, that have formed new host associations with plantation species, as well as non-native insect pests that have been accidentally introduced across the globe (Paine et al. 2011). The rate of increase in the introduction and establishment of non-native pests has increased drastically over the years, largely due to the increase in global trade and travel (Wingfield et al. 2008).

The eucalyptus gall wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae) is a relatively recently discovered non-native insect pest of *Eucalyptus* spp. in plantations. Although native to Australia, it remained unknown and undescribed until its discovery in the Middle East and Mediterranean region in 2000. It has subsequently spread to all continents where *Eucalyptus* spp. are grown commercially, namely Europe, Africa, Asia, South America and North America (Mendel et al. 2004; Paine et al. 2011). Infestation by the wasp results in gall formation on the midribs, petioles and stems of *Eucalyptus*, resulting in deformation and stunting of trees. Severe infestations, especially when plants are young, can result in tree death.

The main management strategy for *L. invasa* is currently focused on host resistance and biological control. Insecticides have been used, but are generally considered ineffective (Kulkarni 2010). For host resistance, various affected countries have tested locally deployed *Eucalyptus* genotypes for susceptibility to *L. invasa* (for example, Thu et al. 2009; Nyeko et al. 2010; Dittrich-Schröder et al. 2012). Results of these studies have shown great variability in susceptibility between and within different *Eucalyptus* spp. and hybrids, and have thus raised the potential to use host resistance as part of a management strategy. However, host resistance alone is likely to be insufficient, because observations in the field have demonstrated that host resistance can be overcome in the presence of high *L. invasa* population levels. It is thus important that host resistance and biological control are used in combination.

Natural enemies of *L. invasa* have been collected in Australia as part of a search of suitable biological control agents. This resulted in the discovery of *Quadrastichus mendeli* (Hymenoptera: Eulophidae), *Selitrichodes kryceri* (Hymenoptera: Eulophidae), as well as a *Megastigmus* sp. (Hymenoptera: Torymidae) (Kim et al. 2008; Protasov et al. 2008; Doğanlar and Hassan 2010). These parasitic wasps were introduced in Israel where they have become fully established. Unfortunately, difficulties with breeding these wasps in quarantine facilities in South Africa has restricted their potential use as biological control agents and this has necessitated the search for other biological control options.

*Selitrichodes neseri* (Hymenoptera: Eulophidae), a parasitic wasp of *L. invasa*, was discovered in Australia in 2010 and it has been reared in quarantine in South Africa to study its biology and host specificity (Kelly et al. 2012). Rearing of *S. neseri* was very successful in quarantine, where it infested over 70% of suitable *L. invasa* galls (unpublished data). Other characteristics that have suggested that *S. neseri* could be an ideal biological control agent include the fact that it has a short developmental time in the gall (mean = 19 days), there is no pre-oviposition period, the adults have a long life span (mean = 26 days when supplemented with honey water), and the parasitoid has the ability to utilize a range of different gall ages. Furthermore, *S. neseri* showed a high level of host specificity when exposed to a number of potential non-target hosts that were phylogenetically similar to or induced galls similar in morphology to those of *L. invasa* (unpublished data).

The first releases of *S. neseri* in the field were made in July 2012 in Zululand, South Africa, a sub-tropical area with high *L. invasa* infestations. Subsequent releases have now been made in all the major regions in South Africa infested with *L. invasa*. Encouragingly, the first *S. neseri* were recovered from the field just two months after the original introduction. Surveys are ongoing to investigate the establishment and spread of *S. neseri*. Research is also underway to consider the influence of temperature and other climatic factors on *S. neseri* survival and establishment, and to better understand its behavior, including host location and resource allocation. Based on the performance of *S. neseri* in quarantine, expectations are that this parasitoid will become an effective biological control agent and that it will contribute towards the global management of *L. invasa*.

## References

Doğanlar, M., and E. Hassan. 2010. Review of Australian Species of *Megastigmus* (Hymenoptera: Torymidae) Associated with *Eucalyptus*, with Descriptions of New Species. Austra. J. Basic Appl. Sci. 4: 5059–5120.

Dittrich-Schröder, G., M. J. Wingfield, B. P. Hurley, and B. Slippers. 2012. Diversity in *Eucalyptus* susceptibility to the gall-forming wasp *Leptocybe invasa*. Agric. For. Entomol. 14: 419–427.

Kulkarni, H. D. 2010. Screening eucalyptus clones against *Leptocybe invasa* Fisher and La sale (Hymenoptera: Eulophidae). Karnataka J. Agric. Sci. 23: 87–90.

Kelly, J., J. La Salle, M. Harney, G. Dittrich-Schröder, and B. Hurley. 2012. *Selitrichodes neseri*, a new parasitoid of the eucalyptus gall wasp *Leptocybe invasa* Fischer & La Salle (Hymenoptera: Eulophidae: Tetrastichinae). Zootaxa 3333: 50–57.

Kim, I., Z. Mendel, A. Protasov, D. Blumenberg, and J. La Salle. 2008. Taxonomy, biology and efficacy of two Autralian parasitoids of the eucalyptus gall wasp, *Leptocybe invasa* Fischer & La Salle (Hymenoptera: Eulophidae: Tetrastichinae). Zootaxa*.* 1910: 1–20.

Mendel, Z., A. Protasov, N. Fischer, and J. La Salle. 2004. Taxonomy and biology of *Leptocybe invasa* gen. & sp. n. (Hymenoptera: Eulophidae), an invasive gall inducer on *Eucalyptus*. Australian J. Entomol. 43: 101–113.

Nyeko, P., K. E. Mutitu, B. O. Otieno, G. N. Ngae, and R. K. Day. 2010. Variations in *Leptocybe invasa* (Hymenoptera: Eulophidae) population intensity and infestation on eucalyptus germplasms in Uganda and Kenya. Int. J. Pest Manage. 56: 137–144.

Paine, T. D., M. J. Steinbauer, and S. A. Lawson. 2011. Native and exotic pests of *Eucalyptus*: A worldwide perspective. Ann. Rev. Entomol. 56: 181–201.

Protasov, A., M. Doĝanlar, J. La Salle, and Z. Mendel. 2008. Occurrence of two local *Megastigmus* species parasitic on the eucalyptus gall wasp *Leptocybe invasa* in Israel and Turkey. Phytoparasitica 36: 449–459.

Thu, P. Q., B. Dell, and T. I. Burgess. 2009. Susceptibility of 18 eucalypt species to the gall wasp *Leptocybe invasa* in the nursery and young plantations in Vietnam. Science Asia 35: 113–117.

Wingfield M. J., B. Sippers, B. P. Hurley, T. A. Coutinho, B. D. Wingfield, and J. Roux. 2008. Eucalypt pests and diseases: growing threats to plantation productivity. Southern Forests 70: 139–144.

# Building BiCEP: a global collaboration for the biological control of eucalypt insect pests

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## Summary

Insect pests of Australian origin threaten the productivity and sustainability of eucalypt plantations worldwide. In the last decade, new pests such as the bronze bug (*Thaumastocoris peregrinus*) Carpintero & Dellapé (Heteroptera: Thaumastocoridae), two gall wasps (*Leptocybe invasa* and *Ophelimus maskelli*) and a lerp psyllid (*Glycaspis brimblecombei*) have emerged as key global pests, while longer-established pests such as the eucalyptus snout beetle (*Gonipterus* species complex) are re-emerging as significant issues in some regions. The speed at which these new pests have emerged, invaded and then spread globally has been taxing the international plantation eucalypt industry's ability to effectively manage them using conventional methods such as deployment of resistant germplasm or insecticidal control. Only biological control has shown potential in effectively managing these pests. In the past, affected countries have managed these programs individually. However, the frequency at which new pests are emerging and the rapidity with which they move around the world means that combating them in isolation is no longer an effective strategy. An Australian Centre for International Agricultural Research (ACIAR) scoping project in 2011-12 demonstrated that the strong industry and professional links needed to underpin an international collaborative approach to biological control are now in place.

The establishment of an alliance (Biological Control of Eucalypt Pests - BiCEP) based in Australia, will provide a focus for developing effective biological control solutions for key pests. This alliance will seek co-funding from the international eucalypt plantation industry and government funding agencies, and will deliver the research and development required to address biological control of eucalypt pests in three categories:

1) Discovery: pests that do not have known, effective biological control agents (e.g. *Thaumastocoris peregrinus*);
2) Application**:** pests with known, but not yet established or industry-evaluated biological control agents (e.g. *Leptocybe invasa, Ophelimus maskelli*);
3) Fine-tuning**:** pests that have established biological control agents but which require better climate/host matching options (e.g. the *Gonipterus* spp. complex, *Glycaspis brimblecombei*).

Membership of BiCEP is being sought from a broad base of countries and regions that have commercial eucalypt plantation industries. A core partnership, based on collaborators in Australia, South America, South Africa and Southeast Asia, is being planned for 2013, with a strategic intent to expand to include growers in Asia, Europe and elsewhere as soon as is practicable. The chief initial focus of BiCEP will be to discover and deploy effective biological control agents for *T. peregrinus*, the key pest issue for industry in South America and South Africa, and which is also on the move globally, having most recently been detected in Italy (2011) and now in New Zealand and Portugal (2012), and with port interceptions reported in Asia in 2012. Other priorities will be to apply and fine-tune the biological control of *L. invasa*, *G. brimblecombei* and the *Gonipterus* spp. complex.

Whilst the initial focus of the research will necessarily be in Australia, BiCEP will also support activities in collaborating countries as appropriate by maintaining flexibility in funding arrangements. Support for students and exchanges of staff will be a high priority.

## Background

There are currently more than 20 million ha of eucalypt plantations established worldwide (Iglesias-Trabado and Wilstermann 2009). The productivity of existing plantations and the development of new plantations to meet world demand for wood fibre are increasingly under threat from a suite of Australian eucalypt insects that have been expanding their global ranges over the past decade. The most serious of these pests that currently threaten plantations and their present distribution are listed in Table 1.

**Table 1:** Current distribution of the 'big five' eucalypt pests around the world and pest status in Australia. * North Africa; ** Recent introduction into South Africa (July 2012).

| **Common name**<br>***Latin name*** | **South America** | **Africa** | **Asia** | **Europe** | **North America** | **Australia (pest status)** |
|---|---|---|---|---|---|---|
| Bronze Bug<br>*Thaumastocoris peregrinus* | + | + | - | + | - | + |
| Blue gum chalcid<br>*Leptocybe invasa* | + | + | + | + | + | - |
| Eucalyptus gall wasp<br>*Ophelimus maskelli* | - | +* | + | + | - | - |
| Red gum lerp psyllid<br>*Glycaspis brimblecombei* | + | +** | - | + | + | - |
| Eucalyptus snout beetle<br>*Gonipterus* spp. Complex | + | + | - | + | + | + |

In addition to these insects, further introductions of new pests are increasingly likely in the future, facilitated by expanding world trade and movement of people. The number of new introductions of Australian insects has risen almost exponentially since 2000 (Murphy et al. in prep; Paine et al. 2011) and this trend is likely to continue over the longer term (Garnas et al. 2012; Wingfield et al. 2008). Once established in one country, movement of pests between countries and continents is also now much more rapid (Garnas et al. 2012). For example, since its initial establishment in Israel in 2000, *L. invasa* has spread within a decade to all continents where eucalypts are grown. There is therefore a critical need for eucalypt growers worldwide, including in Australia, to address the ongoing issue of the emergence of new pests and their rapid movement across the globe.

Biological control is a well-established, highly effective method for controlling exotic pests and has a proven track record around the world. However, efforts directed at eucalypt pests globally have been fragmented, uncoordinated and inefficient in use of resources (Garnas et al. 2012). Establishment of an Australian-based alliance is therefore urgently required to coordinate and carry out the basic research and development program that will provide biological control solutions for the key pests and issues defined above.

The key pests identified in Table 1 will be the focus of the alliance, and fall into three main categories in regard to biological control R&D needs (see Table 2 below).

Table 2: Biological control requirements of the 'big five' eucalypt pests. Category 1 – Discovery; Category 2 – Application; Category 3 – Fine-tuning.

| Category | Pest | Biological Control Effectiveness |
|---|---|---|
| **1** | Bronze Bug<br>*Thaumastocoris peregrinus* | Unknown. |
| **2** | Blue gum chalcid<br>*Leptocybe invasa* | Unknown in industrial scale plantations. |
| **2** | Eucalyptus gall wasp<br>*Ophelimus maskelli* | Unknown in industrial scale plantations. |
| **3** | Red gum lerp psyllid<br>*Glycaspis brimblecombei* | Good, but not in all climatic zones. Better biotype/species matching required. |
| **3** | Eucalyptus snout beetle<br>*Gonipterus* spp. complex | Good, but not in all climatic zones. As well as better biotype matching, cryptic host species may cause parasitoid/biotype mismatches. |

## Conclusions

The global eucalypt plantation industry faces significant challenges from the rapid movement of a growing number of Australian invasive eucalypt insect pests. Management options for such pests are limited. Selection of germplasm for pest resistance is now of reduced effectiveness due to the sheer numbers of pest species invading that have diverse feeding and lifecycle habits. Chemical control is rarely an optiondue to the impracticality of use and prohibitive cost of treating large-scale plantations and limitations associated with certification for sustainability. Only classical biological control offers a relatively low-cost and proven option to manage exotic pests, whilst promoting sustainable forest management to meet market certification requirements. Given the global nature of the problem, an international collaborative approach is now urgently required to provide biological control solutions (see http://bicep.net.au).

## References

Garnas, J., B. Hurley, B. Slippers, and M. Wingfield. 2012. Biological control of forest plantation pests in an interconnected world requires greater international focus. Int. J. Pest Manage. 58: 211–223.

Iglesias-Trabado, G., and D. Wilstermann. 2009. Eucalyptus universalis. Global cultivated eucalypt forests map 2009. http://git-forestry-blog.blogspot.com/2009/10/global-eucalyptus-map-2009-in-buenos.html

Murphy, B. D., H. F. Nahrung, F. R. Wylie, and S. A. Lawson. (in preparation) Pathways for the global invasion of eucalyptus insect pests.

Paine, T. D., M. J. Steinbauer, and S. A. Lawson. 2011. Native and Exotic Pests of Eucalyptus: a Worldwide Perspective. Ann. Rev. Entomol.ogy. 56: 181-201.

Wingfield, M. J., B. Slippers, B. P. Hurley, T. A. Coutinho, B. D. Wingfield, and J. Roux. 2008. Eucalypt pests and diseases: growing threats to plantation productivity. Southern Forests. 70: 139-144.

# Dispersal behaviour of the parasitic wasp *Cotesia urabae*: a biological control agent against gumleaf skeletoniser *Uraba lugens* in New Zealand

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## Abstract

Biological control programs provide a great opportunity to study the ecology of the introduced agents in this new environment. Estimates of the dispersal rate of the biological control agent in its new environment are vital to understanding its relative searching capacity, and to foresee the maximum area that could be covered in a release event. In New Zealand, the solitary endoparasitoid *Cotesia urabae* Austin and Allen (Hymenoptera: Braconidae) was first released in 2011 as a biological control agent for the gum leaf skeletoniser, *Uraba lugens* Walker (Lepidoptera: Nolidae). Our objective was to utilize an experimental approach to quantify the dispersal behaviour of one generation of *C. urabae*. In our experiment, which used sentinel larvae as target hosts, parasitoids dispersed up to 20 m away from the release point and were most successful parasitizing hosts located no more than 5 m from the release point. According to the dispersal model adjusted from the data collected, *Cotesia* would be able to disperse up to 53 m in one release event, which is supported by data following field releases. In addition, significant differences ($P < 0.001$) were found between the different directions tested for dispersal. Our results suggest that wind has a direct effect on the dispersal behaviour of *C. urabae* in the field, showing a clear downwind dispersal.

## Introduction

When introducing a new biological control agent, one of the most interesting and important aspects of their biology to assess is its rate of dispersal (Godfray 1994). This rate of dispersal is understood as the numerical variation in a population due to the natural movement of its individuals (Ricklefs and Miller 2000). There are different mark-release-recapture techniques that are used to estimate the dispersal of insect populations when they are already present in a determined area (Southwood 1978; Walker and Wineriter 1981; Hagler and Jackson 2001). However, when estimating the dispersal of a recently introduced species, it is possible to obviate many steps often required in studies of insect dispersal for established or native species (Castillo et al. 2006). *Cotesia urabae* is a solitary endoparasitoid that was introduced into New Zealand during 2011 as a biological control agent for *Uraba lugens* (Avila et al. 2013), a lepidopteran pest of eucalypt trees. Here, we used *U. lugens* sentinel larvae to assess patterns of initial dispersal for *C. urabae*. The data collected allowed to develop a dispersal model to estimate its dispersal radius. It will help us to understand its dispersal and movement behaviour once released in the environment.

## Materials and Methods

A total of 49 potted *Eucalyptus fastigata* trees (approximately 1 m tall) were used for the experiment. Trees were set out in a randomized block design in concentric circles of fixed distances. One plant was marked and labeled as the release tree (RT) and placed in the center of the field, and starting from this epicenter; six more plants were marked and labeled similarly with their location and placed in a line at a distance of 5, 10, 15, 20, 25 and 30 m from the release. This procedure was made following the eight cardinal points, N, NE, E, SE, S, SW, W and NW. Batches of 3rd – 5th instar *U.*

*lugens* larvae were attached onto leaves on each potted tree. Two hundred larvae were attached in the release tree (50 larvae in each N, E, S and W direction) and 50 more larvae in each of the other 48 trees. One release of 55 adult parasitoids was made (35 females and 20 males) in the epicenter of the six concentric circles. The release was made on May 9th 2011 at 9am; the temperature was 16.5ºC, a relative humidity of 95.5%, an average wind speed of 1 km/h and prevailing winds were from a SW-W direction. Wind speed and its direction were checked 3 and 7 days after the release. Host sentinel larvae were recovered from the field one week after the release and brought back to the laboratory to check for parasitism.

## Results

Host larvae were recovered from the 49 trees used in the experiment, but after rearing *U. lugens* larvae collected from the experiment, parasitism was confirmed only from 17 trees. A final number of 441 *C. urabae* cocoons were recovered from parasitized hosts. We found that *C. urabae* parasitized host larvae up to a distance of 20 m from the epicenter, NE (n=11) and E (n=5), and dispersed in all directions. 77.1% of the parasitoid cocoons recovered were from plants located in a radius no greater than 5 m from the epicenter. Significant differences ($F = 17.35$; $P < 0.001$) were found between the parasitism rate observed and the different distances evaluated, and the parasitism rate was significantly higher (mean 47.6%, overall significance level 0.05) at 5 m from the epicenter. This suggests that a higher level of parasitism occurred on hosts at closer proximity to the epicenter and progressively decreased towards more distant hosts.

A clear dispersal trend to the NE and E directions was also observed. There were significant differences ($F = 5.38$; $P < 0.001$) between the parasitism rate observed and the different directions evaluated, which suggest a clear influence of wind on the dispersal of *C. urabae*, allowing it to have a downwind dispersal, thus, to be more abundant on plants located at NE and E directions. The highest parasitism rate (mean 32.6%, overall significance level of 0.05) was detected at NE direction, whereas at other distances parasitism decreased to 28.9% and 13.8%, at E and N directions respectively. In the other directions, parasitism rate dropped up to 3.9% and no significant differences were detected. According to the data collected from the experiment, it was observed that the model that best describes the dispersal of *C. urabae* was an exponential model which takes the form: $N = e\text{^} (a+b\sqrt{x})$, where $N$ is the number of individuals dispersing to distance $X$; while $a$ and $b$ are constants. By using the parameter's values calculated for the dispersal model, it is predicted that *C. urabae* might disperse up to 53 m in the field.

### Conclusions

Our results show that *C. urabae* did not disperse great distances from its released point, and generally tends to parasitize hosts located no further than 5 m from the epicenter. This suggests that most of the females released may have remained in the release tree. The short dispersal distance observed may be explained by a number of different factors. Several studies had reported the direct impact of weather conditions on insect behaviour after experimental releases (Fink and Volkl 1995; McClure 1990; Weisser et al. 1997). During our experiment, a severe rain event two days after the release may have adversely affected *C. urabae* dispersal. Other studies have also shown that some *Cotesia* species use infochemicals to locate their hosts, where females are attracted to plants with a higher number of host larvae and abundant amount of frass (Ngi-Song and Overholt 1997; Ngi-Song et al. 1996). Therefore, in our experiment, the higher parasitism found at shorter distances could also be the parasitoid response to a higher host density at closer distances to the epicenter, thus causing the aggregation. Wind had a direct effect on the dispersal behavior of *C. urabae* in the field, showing a clear downwind dispersal, in this case to NE and E directions. Similarly to other observations made on insects' dispersal models (Castillo et al. 2006; Sallam et al. 2001; Taylor 1978), and even though the maximum distance of dispersion observed for *C. urabae* was 20 m, the dispersion model adjusted suggests that the parasitoids may disperse to higher distances, and in this case up to 53 m in one release event. This results match with field observations made by Avila et al. (2013) after monitoring *C. urabae* releases made in Auckland in 2011, where cocoons have been found at 50 m and 60 m from the release tree after nearly two and four months of the latest release event.

## References

Avila, G. A., L. A. Berndt, and G. I. Holwell. 2013. First releases and monitoring of the biological control agent *Cotesia urabae* Austin and Allen (Hymenoptera: Braconidae). NZ Entomol. 36: 1–8.

Castillo, A., F. Infante, J. Valle, and J. C. Espinoza. 2006. Dispersión del parasitoide africano *Phymastichus coffea* LaSalle. (Hymenoptera Eulophidae) en un nuevo agroecosistema. Folia Entomol. Mex. 45: 319–327.

Fink, U., and W. Volkl. 1995. The effect of abiotic factors of foraging and oviposition success of the aphid parasitoid, *Aphidius rosae*. Oecologia 103: 371–378.

Godfray, H. C. J. 1994. Parasitoids : behavioral and evolutionary ecology. Princeton University Press, Princeton, NJ, USA

Hagler, J. R., and C. G. Jackson. 2001. Methods for marking insects: Current techniques and future prospects. Ann. Rev. Entomol. 46: 511-543.

McClure, M. S. 1990. Role of Wind, Birds, Deer, and Humans in the Dispersal of Hemlock Woolly Adelgid (Homoptera: Adelgidae). Environ. Entomol. 19: 36–43.

Ngi-Song, A. J., and W. A. Overholt. 1997. Host location and acceptance by *Cotesia flavipes* Cameron and C. *sesamiae* (Cameron) (Hymenoptera: Braconidae), parasitoids of african gramineous stemborers: Role of frass and other host cues. Biological Control 9: 136–142.

Ngi-Song, A. J., W. A. Overholt, and P. G. N. Njagi. 1996. Volatile infochemicals used in host and host habitat location by *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), larval parasitoids of stemborers on graminae. J. Chem. Ecol. 22: 307–323.

Ricklefs, R. E., and G. L. Miller. 2000. Ecology, 4th ed. W.H.Freeman Co Ltd, New York.

Sallam, M. N., W. A. Overholt, and E. Kairu. 2001. Dispersal of the exotic parasitoid *Cotesia flavipes* in a new ecosystem. Entomol. Exp. et Applic. 98: 211–217.

Southwood, T. R. E. 1978. Ecological methods : with particular reference to the study of insect populations 2nd ed, Chapman and Hall, London.

Taylor, R. A. J. 1978. The relationship between density and distance of dispersing insects. Ecol. Entomol. 3: 63–70.

Walker, T. J., and S. A. Wineriter. 1981. Marking Techniques for Recognizing Individual Insects. Florida Entomol 64: 18–29.

Weisser, W. W., W. Volkl, and M. P. Hassell. 1997. The importance of adverse weather conditions for behaviour and population ecology of an aphid parasitoid. J Anim. Ecol. 66: 386–400.

# Biological control of *Sirex noctilio* Fabr. (Hymenoptera: Siricidae) in Chile

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*Sirex noctilio* (Hymenoptera: Siricidae) is one of the most important pests associated to *Pinus* sp. plantations worldwide. In 1980, it was detected in Argentina and, since Chile had extensive monospecific *Pinus radiata* plantations (over 1.5 million ha), one of the most susceptible *Pinus* species to the *S. noctilio* attack. The Servicio Agrícola y Ganadero (SAG) decided to initiate, along with the forest sector, a *S. noctilio* early detection program based on the installation of trap tree, forest prospections and light and funnel traps. In 2011, the pest was detected in a *Pinus* stand located in Comuna of Los Andes, Región de Valparaíso (SAG 2001). It is suspected that the introduction of *S. noctilio* was through woodpackaging, as near the stand is an energy plant that has received a great amount of woodpacking material coming from Europe (Beeche 2005). That same year, pest was detected 1 000 km further south, in Región de Los Lagos. Thus, the control of *S. noctilio* was officially declared by the resolution Nº2.630/01 of SAG. The main objective was to eradicate and contain, and in spite of the extensive efforts to avoid the spread of this pest, it moved to the north and in the year 2006 it was decided to modify the objective of the official control program to containment and suppression. The containment program had the objective of postponing the pest spread to regions that concentrate more than 60% of *P. radiata* plantations in the country through quarantine. The program intended to lower the damage levels of the pest using the parasitic nematode *Deladenus siricidicola* (Nematoda: Tylenchida) and the hymenopteran parasitoids *Megarhyssa nortoni* (Hymenoptera: Ichneumonidae), *Ibalia leucospoides* (Hymenoptera: Ibaliidae) and *Rhyssa persuasoria* (Hymenoptera: Ichneumonidae) (Beeche et al 2012).

*Deladenus siricidicola* was introduced in Chile from Brazil in the 90's through collaboration agreement between the SAG and EMBRAPA. The nematode was cryopreserved. In 2006, the inoculations of trap tree were initiated in the quarantine areas of the Los Lagos and Los Rios Regions. Doses were provided by Controladora de Plagas Forestales S.A. (CPF) laboratory, located in Region del Bio-Bio, to which SAG had authorized the maintenance of the cryopreserved nematode. In 2007, SAG initiated the nematode production at its laboratory located in the city of Osorno, Región de Los Lagos. Nowadays, CPF produces doses to be utilized by the private forest sector, whereas SAG produces doses to inoculate trap tree that the Service installs in small lands and medium forest owners.

Additionally, since 2009, the SAG recovered the nematode from the field in geographical areas where the levels of parasitism were more than 85%, and multiplied it in the laboratory. Thus, in 2012, 97% of the doses were produced with material extracted from the field. Through the years, there was an increase in the number of inoculated trap trees until 2011 except in 2012 (Table 1), due to the fact that, in Region de Los Lagos y Rios, parasitism levels of over 85% were observed and, thus, it was decided to decrease the amount of trap trees installed and reinforced activities in the north area of *Sirex noctilio* distribution. (Región del Maule y Bio-Bio).

Table 1. Numbers of trap trees inoculated with *Deladenus siricidicola* in small lands and medium forest owners.

| | Numbers of inoculated trap tree **/year** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | **TOTAL** |
| **Total** | **521** | **1.052** | **937** | **999** | **1.111** | **1.266** | **965** | **6.851** |

With the aim of complementing the action of *Deladenus siricidicola*, a collection program of *M. nortoni* and *R. persuasoria* was carried out in New Zealand. Between 2004 and 2009, 5 collections were made. All biological material came into post-entry quarantine conditions in SAG laboratories, located in Santiago. The first releases of *M. nortoni* in Chile were carried out in 2006, and to this date, between the private sector and the SAG, releases were made at 48 sites with a total of 1.950 adults. *Rhyssa persuasoria* was obtained in lower quantities, and releases could only take place at two sites (Table 2).

Table 2. Number of parasitoid cores released per region in the period 2006-2012.

| **Region** | ***Megarhyssa nortoni*** | ***Rhyssa persuasoria*** |
|---|---|---|
| El Maule | 1 | 0 |
| El Biobío | 20 | 0 |
| La Araucanía | 10 | 1 |
| Los Rios | 8 | 0 |
| Los Lagos | 8 | 1 |
| Aysén | 1 | 0 |
| **Total** | **48** | **2** |

The biological control program advance is monitored annually by sampling logs (3 per site) from trees naturally infested by *S. noctilio*. The amount of sampled sites has varied between 20 and 40, due to the increase of the quarantined area. The levels of parasitism by *D. siricidicola* have been increasing in all regions where the pest is present. The highest parasitism rates were obtained in Region de los Lagos and Los Rios, areas where inoculations have been done for six years. In the season 2011-2012, in Los Lagos, we observed 100% parasitism and in Los Rios 95%. Meanwhile, in Bio-Bio, after two years of inoculations, we observed 11.5% parasitism and detected the presence of *I. leucospoides,* with control values between 13 and 35%. In addition, we detected the presence of *M. nortoni* between Bio-Bio and Los Lagos Regions.

Finally, it can be said that the biological control program of *S. noctilio* has been successful, since *D. siricidicola* has been established in the country, and the parasitism rates have been increasing from south to north annually. Also, there are two important parasitoids established, *M. nortoni* and *I. leucospoides*, which are exerting a complementary action to the nematode. Nevertheless, it is estimated that the Maule and Bio-Bio Regions will need a longer time to reach similar control levels to those obtained in southern regions, because it has been observed higher population levels than those that occur in the south.

## References

Beéche, M., P. González, A. Sandoval, S. Ide, M. Murillo, L. Jaques, S. Rothmann, H. González, S. Estay, C. Muñoz, R. Ferrada, M. Peralta, C. Bravo, H. Sievert, R.

Canales, V. Torres, M. Zapata, and M. Carrillo. 2005. Informe anual del Subdepartamento de Vigilancia y Control de Plagas Forestales y Exóticas Invasoras, 2004. Servicio Agrícola y Ganadero, Santiago.

Beéche, M., D. Lanfranco, M. Zapata, and C. Ruiz, 2012. Surveillance and Control of the *Sirex noctilio* Wasp: The Chilean Experience, pp. 229-246. *In* B. Slippers, P. de Groot, and M. J. Wingfield, The Sirex Woodwasp and it Fungal Symbiont: Research and Management of a Wordwide Invasive Pest. Springer, Dordrecht, The Netherlands.

SAG (Servicio Agrícola y Ganadero). 2001 Resolución Nº283: Declara el control obligatorio de la avispa de la madera del pino Pinus *Sirex noctilio* Fabricius. Servicio Agricola y Ganadero, Departamento Protección Agrícola, Subdepto. Defensa Agrícola. Chile.

# Biological control of *Sirex* woodwasp in Brazil: 25 years of results and challenges

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The European woodwasp, *Sirex noctilio* (Hymenoptera: Siricidae), was introduced in Brazil in Rio Grande do Sul (1988), Santa Catarina (1989), Paraná (1996), São Paulo (2004) and Minas Gerais (2005) States, (Iede et al. 1988; Iede and Zanetti 2007). Nowadays the pest is present in an area of 1,5 million ha of pine plantation. Most of these plantations use loblolly pine (*Pinus taeda*), following by Pinus elliottii and tropical species like *P. oocarpa*, *P. caribaea*, *P. maximinoi* and *P. tecunumannii*. When the woodwasp was introduced (1988) the stands were planted at high density and inadequate forest management. Continous monoculture contributed also for the pest dispersal. The introduction of the pest changed the pine silviculture in Brazil. Before the introduction of the European woodwasp, farmers and industries were concerned only about the control of cut ants in the pine stands and the forest management programs did not involved the forest integrated pest management.

*Sirex* is a plastic species. It lives in temperate climate, under Mediterranean climate, and under subtropical climate. In South America, the first record was in *Pinus taeda and P. elliottii* stands at the Department (State) of Cerro Largo in Uruguay in 1980. The insect spread rapidly to the pine plantations in the country. In 1985, it was detected in *P. taeda* stands at Argentina and dispersed to several provinces. In 1991, it was first detected in the Argentinian Patagonian Andes threatening stands of little economic significance, but of strategic ecological importance and in 2000 in Chile (COSAVE 2001).

The Integrated Pest Management (IPM) Program for *S. noctilio* in Brazil is similar to the programs used in different countries where the pest is present, but enforced with different intensity. The National *Sirex* Control Program include the following measures: 1) monitoring for early detection and to know the dispersal of the pest, using trap trees and sequential sampling; 2) adopting prevention strategies to improve the phytosanitary conditions of forest stands using silvicultural practices, especially thinning of overstocked stands, to minimize the attacks; 3) adopting quarantine strategies to control and slow down dispersal; 4) developing a biological control program with the nematode and parasitoids introduction, to increase the range of natural enemies; 5) publicizing, using the media and the researchers involved, in a vast training program for technical personnel and forest producers to improve specific abilities and give information to the society (Iede et al. 1998; 2000b).

The experience shows that the monitoring and early detection are among the main prevention measures adopted all over the country. Monitoring is made with stressed trees groups using an herbicides to become trees attractive to the pest. If *Sirex* detection is made when the percentage of attacked trees are less than 1% and the nematode release is made immediately, it is possible to avoid economic losses, because there are enough time to the nematode reaches high levels of parasitism.

The first introduction of the nematode in Brazil was done by Embrapa Forestry in 1989/1990, from CSIRO, Australia. Unfortunately the nematode became defective, because it was rearing in laboratory condition for more than twenty 20 years in Australia. Two strategies were used for the problem solution: 1) importing, in 1994 the Kamona strain re-isolated in Tamania from CSIRO, Australia and released in 1995; 2) re-isolated a new strains from the field in Brazil, where the nematode had a good efficiency, in Encruzilhada county, probably due the adaptation to the local conditions (Bedding and Iede 2005). New strains has been isolated and released every year since

1995. Every year, thousands of *Sirex* adults are evaluated for the nematode parasitism and the levels are near 100%. In the year 2000, 3036 insects from 68 farms of a company was evaluated for nematode parasitism level and the average was 77%. Embrapa’s mass rearing laboratory has a capacity to produce about 1500 nematode doses weekly.

*Ibalia leucospoides* (Hymenoptera: Iballidae), a parasitoid of the woodwasp eggs and early stages of the larvae, was accidentally introduced together with the pest and it is present in the whole pest dispersion area. It has an average parasitism of 23% (ranging 4-45%) and a high capacity of establishment. The parasitoid was detected in 1990, controlling up to 29,1% of the pest (Carvalho 1993). Nowadays the parasitoid occurs in nearly all areas where its host is present. In some cases it did a mass rearing in laboratory and later released in fields by reforestation companies, with the objective of introducing the parasitoid in areas where it was not yet present or in the attempt to increase parasitism rates.

In Brazil, the program should be complemented by the introduction of *Megarhyssa nortoni* and *Rhyssa persuasoria* (Hymenoptera: Ichneumonidae), both imported from Tasmania, Australia. The introductions were made in 1996 and 1997, through a cooperative project of Embrapa Forestry, CSIRO, the International Institute of Biological Control (CABI-Bioscience) and the USDA Forest Service. In the 1996 shipments, 77 *M. nortoni* adults arrived alive in Brazil (Iede et al. 2000a). They were kept under quarantine in chambers with controlled humidity and temperature, where 1.8 m long, 0.25 cm diameter *Pinus taeda* billets with *S. noctilio* larvae were offered to them. Billets were collected in Brazil by the end of August, about 45-60 days before parasitoids arrived and it was kept in a cold chamber at 12$^o$C, to synchronize the life cycle of *S. noctilio* with parasitoid (Iede et al. 2000b). In 1997, only 9 females and 4 males of *M. nortoni* and 9 females of *R. persuasoria* were imported. The first generation of *M. nortoni* emerged in 1997 and resulted in 88 females and 45 males, of which 18 mated females were release in the field. The second generation of *M. nortoni* yielded 218 males and 101 females, of which, 136 males and 97 females were released in the field. In 1999, only 50 males and 31 females emerged. The 1998 generation of *R. persuasoria* yielded 18 males and 19 females, of which 2 males and 10 females were released in the field. In 1998, 40 females and 20 males emerged. The establishment of these species at the release areas was not confirmed until now (Iede et al. 2000a).

*S. noctilio* became under control in Brazil since 1995 when it was introduced the Kamona strain, from Tasmania, associated to the use of parasitoïd *I. leucospoides*, silviculture control (thinnings), early detection with trap trees and monitoring using sequential sampling.

## References

Bedding, R., and E. T. Iede. 2005. Application of *Beddingia siricidicola* for *Sirex* woodwasp control. In: Grewal, P. S.; Ehlers, R.-U.; Shapiro-Ilan, D. I. Nematodes as biocontrol agents. Wallingford: CAB International. p. 385-397.

Carvalho, A, G. 1993. Aspectos biológicos de *Ibalia leucospoides* (Hockewarth), (Hymenoptera, Ibaliidae). Anais da Conferência Regional da Vespa-da-Madeira, *Sirex noctilio*, na América do Sul. Colombo: EMBRAPA/FAO/USA/FUNCEMA.

COSAVE. 2001. Informe sobre a ocorrência de *Sirex noctilio* na região do COSAVE. Comitê de Sanidade Vegetal do Cone Sul. Série Técnica. Relatório técnico 001/00.

Iede, E. T., and R. ZanettiI. 2007. Ocorrência e recomendações para o manejo de *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) em plantios de *Pinus patula* (Pinaceae) em Minas Gerais, Brasil. Rev. Brasil. Entomol. 51: 529-531.

Iede E. T., S. R. C. Penteado, and J. C. Bisol. 1988. Primeiro registro de ataque de *Sirex noctilio* em *Pinus taeda* no Brasil. CNPF/Embrapa. Circular Técnica. 20. 1-12.

Iede E. T., S. R. C. Penteado, and E. G. Schaitza. 1998. *Sirex noctilio* in Brazil: detection, evaluation, and control. In: Proceedings of a conference: Training in the control of *Sirex noctilio* by the use of natural enemies,Embrapa/ USDA-FS Colombo, Brazil. 45-52 .

Iede E. T., S. R. C. Penteado, S. Murphy, D. Haugen, and W. Reis Filho. 2000a. Introduction and release of rhyssines to control *Sirex noctilio* in Brazil. Anais do XXI International Congress of Entomology. Foz do Iguaçu. Embrapa Soja. Londrina-PR.

Iede E. T., S. R. C. Penteado, W. Reis Filho, and E. G. Schaitza. 2000b. Situação atual do Programa de Manejo Integrado de *Sirex noctilio* no Brasil. Série Técnica IPEF. 13:11-20.

# Session 4: Classical Biological Control for the Protection of Biodiversity

## Overview of insect biological control in natural systems: past contributions and future challenges

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Both invasive insects and plants can threaten the biodiversity of the natural ecosystems they invade (Van Driesche et al. 2010) and while cases of weed biological control for the protection of nature (49) currently outnumber those against invasive insects (21), many important instances of the latter exist and are the focus of this symposium. Biological control of invasive insects in natural systems has been achieved in a variety of habitats, but especially in forests and on islands (Table 1). In aquatic and wetland habitats, there have been only a few cases of insect biological control, most noticeably the successful protection of white mangrove, *Laguncularia racemosa* (L.) C.F. Gaertn. (Combretaceae), on the Galápagos Islands from destruction by the cottony cushion scale, *Icerya purchasi* Maskell (Hemiptera: Monophlebidae) (Calderón Alvarez et al. 2012). The largest number of cases of use of biological control in forests has been in the temperate forests of North America, in part due to a large influx of European insects there and the similarity of climates. While older (pre 1990s) forest pest biological control projects were done to protect wood as a commodity, many of them also contributed to protecting native plants. More recent projects to save native trees from lethal invasive insects have seen forest protection as their primary purpose. Island ecosystems often harbor many endemic species that can be attacked by polyphagous invasive insects. Biological control on islands can reduce damage of invaders to native plants and their specialized native herbivores. Presentations in this symposium discuss examples from various native forest and island systems where biological control has protected biodiversity. Some are completed and successful; others are still in progress, with their ultimate outcome still unknown. Brief descriptions follow:

***Erythrina gall wasp***. The gall wasp *Quadrastichus erythrinae* Kim (Hymenoptera: Eulophidae) devastated stands of the native wiliwili tree, *Erythrina sandwicensis* Degener (Fabaceae) in Hawii (Kim et al. 2004). This tree is an endemic keystone species in lowland Hawaiian forest, one of the most endangered ecosystems in the world (www.hear.org). In Africa, the parasitoid *Eurytoma erythrinae* Gates and Delvare (Hymenoptera: Eurytomidae) was found attacking *Quadrastichus* spp. (Gates and Delvare 2008), and after its release and establishment, it spread rapidly, greatly reducing *E. erythrinae* densities and lowering risk to this native tree.

***Icerya scales.*** *Icerya* scales have invaded many islands (Galápagos, Seychelles, Ascension, parts of Micronesia) and attacked native plants. On the Galápagos, *I. purchasi* damaged many native plants, often ones with restricted distributions (Causton 2001; Causton et al. 2006). This scale also harmed rare insects dependent on attacked plants (e.g. Roque-Albelo 2003). This scale has now been suppressed on the Galápagos by *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) (Calderón Alvarez et al. 2012).

***Orthezia scale.*** *Orthezia insignis* Browne (Hemiptera: Ortheziidae) invaded St. Helena and by 1992, was killing endangered, endemic gumwood trees, *Commidendrum robustum* DC (Asteraceae) (Fowler 2004). *Hyperaspis pantherina* Fürsch (Coleoptera: Coccinellidae) was released in 1993, and by 1995 scale density had decreased by 97%, ending scale-caused tree mortality (Fowler 2004). Saving this tree protects the newly restored Millenium Forest (St. Helena National Trust 2009), which seeks to restore native gumwood forest to 50% of the island (Fowler 2004; Anon. 2009; Joint Nature Conservation Committee 2009). Endemic *Nesiotes* weevils (Coleoptera: Curculionidae) associated with gumwoods also benefit from protection of their hosts (Joint Nature Conservation Committee 2009).

***Wax scales in Queensland forests.*** In Australia, the exotic wax scales, *Ceroplastes destructor* Newstead and *Ceroplastes rubens* Maskell (Hemiptera: Coccidae) have damaged native forest plants. Honeydew from scales benefitted invasive ants, which compromised native butterfly/ant mutualisms. Biological control of these scales (for crop protection) benefitted native forest species (Waterhouse and Sands 2001; Van Driesche et al. 2010). In woodlands, *C. destructor* damaged *Pittosporum undulatum* Vent., (Pittosporaceae); in rainforests its hosts included *Auranticarpa rhombifolia* Cayzer et al., *Hymenosporum flavum* F. Muell. (Pittosporaceae), *Syzigium* spp., *Syzigium australe* (J.C. Wendl. ex Link) B. Hyland, and *Acmena smithii* (Poir.) Merr. & L.M. Perry (Myrtaceae) (Van Driesche et al. 2011). Two parasitoids – *Anicetus communis* (Annecke) and *A. nyasicus* (Compere) (Hymenoptera: Encyrtidae) – provided complete biological control (Sands et al. 1986). Infested native plants regained vigor and less honeydew reduced numbers of exotic ants. Several native ants have reappeared, benefitting ant-tended butterflies, such as *Hypochrysops miskini* (Waterhouse) and *Pseudodipsas cephenes* Hewitson (Lepidoptera: Lycaenidae) (D. Sands personal communication, in Van Driesche et al. 2011).

***Cycad scale***. On Guam, the invasive scale *Aulacaspis yasumatsui* Takagi (Hemiptera: Diaspididae) destroyed native cycads, the ecological dominants of limestone cycad forests. This scale, in combination with two invasive lepidopterans – *Chilades pandava* (Horsfield) (Lepidoptera: Lycaenidae) and the leafminer *Erechthias* sp. (Lepidoptera: Tineaidae) (Moore et al. 2005; Marler and Muniappan 2006) – killed 60-90% of cycads in some areas from 2004 to 2008, placing *Cycad micronesica* K.D. Hill (Cycadaceae) on the IUCN Red List (Marler et al. 2006). *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae) was introduced from Hawaii, established, and suppressed the scale on mature cycads but not seedlings, which continue to die at elevated rates, pending discovery of more effective biological control agents.

***Emerald ash borer*****.** *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) invaded North America on wooden packing material and is originally from northeastern China, causing >80% mortality of several *Fraxinus* spp. (Olecaceae), which dominant riparian habitats (MacFarlane and Meyer 2005). The beetle also threatened rare species such as pumpkin ash, *Fraxinus profunda* (Bush) Bush (L. Bauer, personal communication) and 21 monophagous moths that depend on ash (Wagner 2007). Three parasitoids, *Oobius agrili* Zhang et al*.* (Hymenoptera: Encyrtidae), *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), and *Spathius agrili* Yang (Hymenoptera: Braconidae), from China were collected, screened for host specificity, released, and recovered (Bauer et al*.* 2007) and their impact is presently being evaluated (Duan et al. 2012).

***Gold spotted oak borer***. *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae) is invasive from Arizona, USA into oak savannahs of California, where it has killed large numbers of oaks. Biological control is being investigated as a potential means to reduce oak death rates and preserve this distinctive California habitat (Center for Invasive Species Research 2012).

***Hemlock woolly adelgid***. In eastern North America, the Asian species *Adelges tsugae* Annand (Hemiptera: Adelgidae) has caused high mortality to eastern hemlock, *Tsuga canadensis* Carrière (Pinaceae) (Evans 2002) inducing vegetation changes detrimental to stream insects (Snyder et al. 2002) and brook trout, *Salvelinus fontinalis* (Mitchell) (Salmoniformes: Salmonidae) (Ross et al. 2003). Specialized predatory beetles have been introduced against it from China, Japan, and the western United States, including *Laricobius* nigrinus Fender (Coleoptera: Derodontidae), and several Asian coccinellids (e.g. Lu and Montgomery 2001; Zilahi-Balogh et al. 2003). *Laricobius nigrinus* is well established at a number of locations and its populations have increased to levels causing significant mortality (Mausel et al. 2010; McDonald, personal communication).

***Winter moth***. *Operophtera brumata* (L.) (Lepidoptera: Geometridae) is a European defoliator of hardwood trees in North America and has been brought under control previously in Nova Scotia (Embree 1971) and British Columbia (Embree and Otvos 1984) and is currently the subject of a third attempt in Massachusetts (J. Elkinton, personal communication).

**Table 1: Classical insect biological control for the protection of nature ecosystems.**

| ***Target species*** | ***Host Plant*** | ***Biome affected (X)*** | | | | ***Outcome$^a$ (✓)*** | | | ***Location/ comments*** | ***References*** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ***TF*** | ***TR F*** | ***C*** | ***OI*** | ***C*** | ***P*** | ***IP*** | | |
| *Adelges tsugae* Annand (hemlock woolly adelgid) | hemlock | *X* | | | | | | ✓ | eastern USA | Evans 2002, Lu and Montgomery 2001,Zilahi-Balogh et al. 2003, Mausel et al. 2010 |
| *Agrilus planipennis* (emerald ash borer) | ash | *X* | | | | | | ✓ | Agents established; Evaluated in prog. | Bauer et al. 2007 |
| *Aulacaspis yasumatsui* (cycad scale) | cycads | | | | *X* | | ✓ | | Guam; control on mature plants only | Ross Miller and Aubrey Moore (personal communication) |
| *Cinara cupressivora* (cypress aphid) | cypress | | *X* | | | | ✓*1* | | East/South Africa<br>1- control due to several factors | |
| *Ceroplastes destructor* (white wax scale) | diverse plants | | *X* | | | ✓ | | | Queensland, Australia | Sands et al. 1986 |
| *Ceroplastes rubens* (pink wax scale) | diverse plants | | *X* | | | | ✓*1* | | Queensland, Australia<br>1-controlled on some native hosts | Waterhouse and Sands 2001 |
| *Coleophora laricella* (larch case bearer) | larch | *X* | | | | ✓ | | | North America | Ryan 1990 |
| *Dendroctonus micans* (spruce beetle) | spruce | *X* | | | | ✓ | | | Western Europe & Caucasus region | Grégoire 1988, Fielding and Evans 1997 |
| *Diprion similis* (pine sawfly) | pine | *X* | | | | ✓ | | | North America | McGugan and Coppel 1962 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Dryocosmus kuriphilus* (chestnut gall wasp) | chestnut | *X* | | | | ✓ ***1*** | | ✓**2** | 1-Japan, USA<br>2- Europe | Moriya et al. 2003,Quacchia et al., 2008 |
| *Gilpinia hercyniae* (European spruce sawfly) | spruce | *X* | | | | ✓ | | | North America | Magasi and Syme 1984 |
| *Homalodisca vitripennis* (glassy winged sharpshooter) | diverse plants | | | | *X* | ✓ | | | Tahiti; | Grandgirard et al. 2009 |
| *Icerya aegyptiaca* | diverse plants | | | | *X* | ✓ | | | Micronesian islands | Brancatini and Sands 1997 |
| *Icerya purchasi* (cottony cushion scale) | diverse plants | | | | *X* | ✓ | | | Ascension Island; Galapagos | Calderón Alvarez et al. 2012; Fowler, unpub.; Ashmole and Ashmole, 1997 |
| *Icerya seychellarum* | diverse plants | | | | *X* | ✓ | | | Seychelles | Johnson and Threadgold1999, K. Beaver, personal communication |
| *Jamella australiae* | *Pandanus tectorius* | | | *X* | | ✓ | | | southern Queensland, Australia | Smith and Smith 2000 |
| *Lymantria dispar* (gypsy moth) | oak | *X* | | | | | ✓ | | Successful control in New England (USA) | Van Driesche et al. 1996 |
| *Metamasius callizona* (bromeliad weevil) | bromeliads | | *X* | | | | | ✓ | Florida (USA) | Cave 2008 |
| *Operophtera brumata* (winter moth) | oak | *X* | | | | ✓ ***1*** | | ✓**2** | 1-Nova Scotia, British Columbia<br>2-Massachusetts | Embree 1971, Embree and Otvos 1984 |
| *Orthezia insignis* | gumwood trees | | | | *X* | ✓ | | | St. Helena | Fowler 2004 |
| *Paratachardina pseudolobata* (lobate lac scale) | diverse plants | | *X* | | | | | ✓ | Florida (USA) | Pemberton 2003 |
| *Quadrastichus erythrinae* (erythrina gall wasp) | *Erythrina* spp. | | *X* | | | | | ✓ | Hawaii; agents being evaluated | Gates and Delvare 2008 |

[a]Degree of control achieved: C-complete control, P-partial control, IP- in progress

## References

Anonymous. 2009. Scrubwood regeneration website. http://home.swipnet.se/~w-17282/endemic/scrubwood.html (accessed 21 May 2012).

Ashmole, N. P. and M. J. Ashmole. 1997. The land fauna of Ascension Island: new data from caves and lava flows, and a reconstruction of the prehistoric ecosystem. J. Biogeogr. 24: 549-589.

Bauer L. S., H. Liu, J. R. Gould, and R. C. Reardon. 2007. Progress on biological control of the emerald ash borer in North America. Biocontrol News Inform. 28(3), 51N–54N.

Brancatini, V.A., and D. P. A. Sands. 1997. Control or no control of breadfruit mealybug (*Icerya aegyptiaca*): selecting the “right” species of *Rodolia*. p. 56. *In* D.F. Waterhouse, and P. Ferrar (eds.). Second Workshop on Biological Control in the Pacific at Nadi, Fiji in 1995. South Pacific Commission, Noumea, New Caledonia.

Calderón Alvarez, C., C. E. Causton, M. S. Hoddle, C. D. Hoddle, R. Van Driesche, and E. J. Stanek. 2012. Monitoring the effects of *Rodolia cardinalis* on *Icerya purchasi* populations on the Galapagos Islands. BioControl 57: 167–179.

Causton, C. E., 2001. Dossier on *Rodolia cardinalis* Mulsant (Coccinellidae: Cocinellinae), a potential biological control agent for the cottony cushion scale, *Icerya purchasi* Maskell (Margarodidae). Charles Darwin Research Station, Galápagos Islands.

Causton, C. E., S. B. Peck, B. J. Sinclair, L. Roque-Albelo, C. J. Hodgson, and B. Landry. 2006. Alien insects: threats and implications for the conservation of the Galápagos Islands. Ann. Entomol. Soc. Amer. 99: 121–143.

Cave, R. D. 2008. Biological control of the Mexican bromeliad weevil. Biocontrol News and Inform. 29: 1N-2N.

Center for Invasive Species Research (University of CA, Riverside) 2012. The Goldspotted Oak Borer (*Agrilus auroguttatus*) Schaeffer (Coleoptera: Buprestidae). http://cisr.ucr.edu/goldspotted_oak_borer.html (accessed 21 May 2012).

Embree, D.G., 1971. *Operophtera brumata* (L.), winter moth (Lepidoptera: Geometridae) pp. 167-175. *In* Anonymous. Biological Control Programmes against Insects and Weeds in Canada, 1959-1968. Commonwealth Agricultural Bureaux, Farnham Royal, UK.

Embree, D. G. and I. S. Otvos. 1984. *Operophtera brumata* (L.) winter moth (Lepidoptera: Geometridae), pp. 353–357. *In* Kelleher, J. S. and M. A. Hulme (eds.). Biological Control Programmes Against Insects and Weeds in Canada, 1969-1980. Commonwealth Agricultural Bureaux, Slough, U.K.

Evans, R.A., 2002. An ecosystem unraveling? pp. 23-33. *In* Onken, B., R. Reardon, J. Lashomb, (eds.). Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium, February 5-7, 2002. East Brunswick, New Jersey, The State University of New Jersey, Rutgers, New Jersey, USA

Fielding, N. J., and H. F. Evans. 1997. Biological control of *Dendroctonus micans* (Scolytidae) in Great Britain. Biocontrol News and Inform. 18(2): 51N-60N.

Fowler, S. V. 2004. Biological control of an exotic scale, *Orthezia insignis* Browne (Homoptera: Ortheziidae), saves the endemic gumwood tree, *Commidendrum robustum* (Roxb.) DC. (Asteraceae) on the island of St. Helena. Biol. Control 29: 367–374.

Gates, M., and G. Delvare. 2008. A new species of *Eurytoma* (Hymenoptera: Eurytomidae) attacking *Quadrastichus* spp. (Hymenoptera: Eulophidae) galling *Erythrina* spp. (Fabaceae), with a summary of African *Eurytoma* biology and species checklist. Zootaxa 1751: 1–24.

Grandgirard, J., M. S. Hoddle, J. N. Petit, G. K. Roderick, and N. Davies. 2009. Classical biological control of the glassy-winged sharpshooter, *Homalodisca vitripennis*, the egg parasitoid *Gonatocerus ashmeadi* in the Society, Marquesas and Austral archipelagos of French Polynesia. Biol. Control 48: 155–163.

Grégoire, J. C. 1988. The greater European spruce beetle, pp. 455–478. *In* Berryman, A. A. (ed.). Dynamics of Forest Insect Populations. Plenum, NY.

Johnson, S., and R. Threadgold. 1999. Report on the monitoring and status of the coccid (*Icerya seychellarum*) on Aldabra from 1980 to 1999. Unpublished report, Seychelles Islands Foundation.

Joint Nature Conservation Committee, 2009. 15: St Helena http://www.jncc.gov.uk/pdf/OT_StHelena.pdf (accessed 21 May 2012).

Kim, I. K., G. Delvare, and J. La Salle. 2004. A new species of *Quadrastichus* (Hymenoptera: Eulophidae): A gall-inducing pest on *Erythrina* (Fabaceae). J. Hymenopt. Res. 13: 243–249.

Lu, W., and M. E. Montgomery. 2001. Oviposition, development and feeding of *Scymnus* (*Neopullus*) *sinuanodulus* (Coleoptera: Coccinellidae): a predator of *Adelges tsugae* (Homoptera: Adelgidae). Ann. Entomol. Soc. Amer. 94: 64–70.

MacFarlane, D. W., and S. P. Meyer. 2005. Characteristics and distribution of potential ash tree hosts for emerald ash borer. Forest Ecol. Manag. 213: 15–24.

Magasi, L. P., and P. D. Syme. 1984. *Gilpinia hercyniae* (Hartig), European spruce sawfly (Hymenoptera: Diprionidae), pp. 295-297. *In* Kelleher, J. S. and M. A. Hulme, M. A. (Eds). Biological Control Programmes against Insects and Weeds in Canada 1969-1980*.* Commonwealth Agricultural Bureaux Franham Royal, UK,

Marler, T. E., and R. Muniappan. 2006. Pests of *Cycas micronesica* leaf, stem, and male reproductive tissues with notes on current threat status. Micronesica 39(1), 1–9. http://www.uog.edu/up/micronesica/dynamicdata/assetmanager/images/pdf/marler.pdf (accessed 21 May 2012).

Marler, T., J. Haynes, and A. Lindstrom. 2006. *Cycas micronesica*. In: IUCN 2009. IUCN Red List of Threatened Species. Version 2009.1. http://www.iucnredlist.org/details/61316/0/ (accessed 21 May 2012).

Mausel, D. L., S. M. Salom, L. T. Kok, and G. A. Davis. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the eastern United States. Environ. Entomol. 39: 440–448.

McGugan, B. M., and H. C. Coppel. 1962. Biological control of forest insects—1910-1958, pp. 35-127. *In* Anon. A Review of the Biological Control Attempts against Insects and Weeds in Canada. Commonwealth Agricultural Bureaux Franham Royal, UK.

Moore, A., T. Marler, R. H. Miller, and R. Muniappan. 2005. Biological control of cycad *Aulacaspis* scale on Guam. Cycad Newsletter 28(5): 6–8. http://www.cycadsg.org/publications/CAS/TCS-Moore-etal.pdf (accessed 21 May 2012).

Moriya, S., M. Shiga, and I. Adachi. 2003. Classical biological control of the chestnut gall wasp in Japan, pp. 407-415. *In* Van Driesche, R. G. (ed.). Proceedings of the 1st International Symposium on Biological Control of Arthropods, Honolulu, Hawaii, 14-18 January 2002, USDA Forest Service, Morgantown, WV.

Pemberton, R. W. 2003. Potential for biological control for control of the lobate lac scale, *Paratachardina lobata lobata* (Chamberlin) (Hemiptera: Kerridae). Fla. Entomol. 86: 354–361.

Quacchia, A., S. Moriya, G. Bosio, I. Scapin, and A. Alma. 2008. Rearing, release and settlement prospect in Italy of *Torymus sinensis*, the biological control agent of the chestnut gall wasp *Dryocosmus kuriphilus*. BioControl 53: 829–839.

Roque-Albelo, L. 2003. Population decline of Galapagos endemic Lepidoptera on Volcan Alcedo (Isabela island, Galápagos Islands, Ecuador): an effect of the introduction of the cottony cushion scale? Bull. Instit. R. Sc. N. B-E. 73: 1–4.

Ross, R.M., R. M. Bennett, C. D. Snyder, J. A. Young, D. R. Smith, and D. P. Lemarie. 2003. Influence of eastern hemlock (*Tsuga canadensis* L.) on fish community structure and function in headwater streams of the Delaware River basin. Ecol. Freshw. Fish 11: 1–6.

Ryan, R. B. 1990. Evaluation of biological control: introduced parasites of larch casebearer (Lepidoptera: Coleophoridae) in Oregon. Environ. Entomol. 19: 1873–1881.

Sands, D. P. A., R. G. Lukins, and G. J. Snowball. 1986. Agents introduced into Australia for biological control of *Gascardia destructor* (Newstead) (Hemiptera: Coccideae). J. Aust. Entomol. Soc. 25: 51–59.

Smith, N. J. and D. Smith. 2000. Studies on the flatid *Jamella australiae* Kirkaldy causing dieback in *Pandanus tectorius* var. *pedunculatus* (A.Br.) Domin on the

Sunshine and Gold Coasts in Southeast Queensland. Gen. Appl. Entomol. 29: 11–20.

Snyder, C.D., J. A. Young, D. P. Lemarié, and D. R. Smith. 2002. Influence of eastern hemlock (*Tsuga canadensis*) forests on aquatic invertebrate assemblages in headwater streams. Can. J. Fish. Aquat. Sci. 59: 262–275.

St. Helena National Trust 2009. St Helena's Millennium Forest: A symbol of small islands fight to defend fragile eco-systems http://www.nationaltrust.org.sh/millennium_forest.html (accessed 21 May 2012).

Van Driesche, R. G., S. Healy, and R. Reardon. 1996. Biological control of arthropod pests of the northeastern and north central forests in the United States: A review and recommendations. United States Department of Agriculture, Forest Service, Morgantown, West Virginia, FHTET-96-19.

Van Driesche, R. G., R. I. Carruthers, T. Center, M. S. Hoddle, J. Hough-Goldstein, L. Morin, L. Smith, D. L. Wagner et al. 2010. Classical biological control for the protection of natural ecosystems. Biol. Control Suppl. 1, S2-S33.

Wagner D. L., 2007. Emerald ash borer threatens ash-feeding Lepidoptera. News. Lepidop. Soc. 49:10–11.

Waterhouse, D. F. and D. P. A. Sands. 2001. Classical Biological Control of Arthropods in Australia. Australian Centre for International Agricultural Research, Canberra, Australia.

Zilahi-Balogh, G. M. G., L. M. Humble, A. B. Lamb, S. M. Salom, and L. T. Kok. 2003. Seasonal abundance and synchrony between *Laricobius nigrinus* (Coleoptera: Derodontidae) and its prey, the hemlock woolly adelgid (Hemiptera: Adelgidae). Can. Entomol. 135: 103–115.

# Biological control of the Erythrina gall wasp: protecting a native keystone forest species in Hawaii

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The Erythrina gall wasp, *Quadrastichus erythrinae* Kim (Hymenoptera: Eulophidae) (Figure 1), recently invaded a wide swath of Asian and Pacific countries, causing severe damage to several species of *Erythrina* (Fabaceae) trees. It poses an imminent threat to native *Erythrina* species in Latin America, Asia, Australia and the Pacific. In Hawaii, besides directly killing a number of exotic and valuable ornamental trees, it also threatens the native *Erythrina sandwicensis* Degener, which is a keystone tree species in the Islands' few remaining low elevation dryland forests.

Figure 1. Erythrina Gall Wasp, *Quadrastichus erythrinae*

Hawaii has more endangered species, and in fact entire endangered ecosystems, than anywhere in the world. Dryland forest ecosystems have been decimated by land development, urbanization, and "invasional meltdown" characterized by an influx of exotic weeds, ungulates, rodents, and arthropods. It has been estimated that 95% of dryland forests in Hawaii have disappeared in the past 100 years, and 25% of its species are on the Federal endangered species list. *Erythrina sandwicensis* (or wiliwili) is an iconic Hawaiian dryland forest tree (Figure 2), and one of the few deciduous native trees in the Islands. It has strong cultural associations with indigenous Hawaiian people, historically being used for fishing and boating gear, surfboards, and lei.

Figure 2. *Erythrina sandwicensis*

The Erythrina gall wasp oviposits into the growing tips, leaves, shoots, and flowers of trees and causes deformation of plant tissues and rapid death of even large trees. First found in Hawaii in 2005 (Heu et al. 2006), the gall wasp spread rapidly throughout the Islands, and quickly killed many large specimens of *Erythrina variegata* L. (Fabaceae) and other exotic *Erythrina* species that are frequently planted as shade and ornamental trees in residential and commercial areas. *Quadrastichus erythrinae* also started attacking and causing serious deformations in the native *E. sandwicensis*, which was already a beleaguered species.

Although the exact geographic origins of the gall wasps are unknown, host plant relationships and molecular analyses of genetic markers indicate a single invasive species with low genetic diversity that recently emerged from sub-Saharan Africa (Messing et al. 2009; Rubinoff et al. 2010). Entomologists from the University of Hawaii and the Hawaii Dept. of Agriculture conducted explorations for natural enemies in Benin, Kenya, Madagascar, Mozambique, South Africa and Tanzania from 2005–2008. A considerable number of parasitoid species were collected in different countries and from various gall formers on a diversity of *Erythrina* plants. Two of these parasitoids, *Eurytoma erythrinae* Gates and Delvare (Hymenoptera: Eurytomidae) and *Aprostocetus nitens* Prinsloo & Kelly (Hymenoptera: Eulophidae), were imported to Hawaii, where rearing colonies were established in quarantine in Honolulu (Figure 3).

Figure 3. *Eurytoma erythrinae* (left); and *Aprostocetus nitens* (right)

After appropriate non-target testing in quarantine showed minimal risk to both Hawaiian native and beneficial species, State and Federal permits were obtained for the field release of *E. erythrinae*. The parasitoid rapidly established and spread in Hawaii, where initial results for the control of the gall wasp on the native *E. sandwicensis* appear promising (Figure 4). We discuss the results of this project in terms of the ecology of the *Erythrina* system, as well as the politics of the practice of biological control in Hawaii.

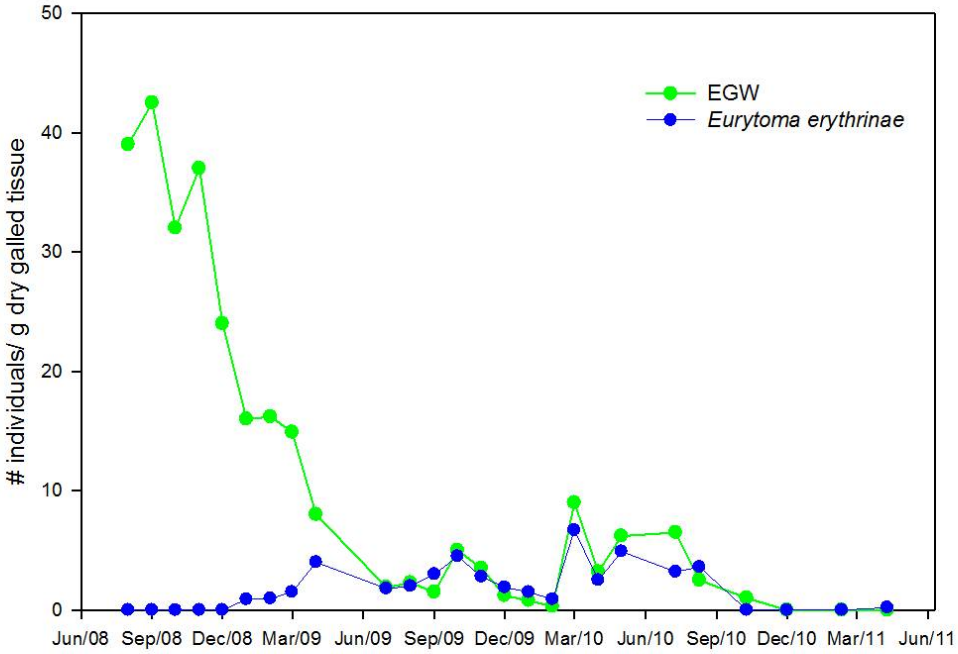

Figure 4. Population density of the Erythrina gall wasp declined following introduction of the parasitoid *Eurytoma erythrinae*.

## References

Heu, R., D. Tsuda, W. Nagamine, J. Yalemar, and T. Suh. 2006. *Erythrina Gall Wasp, Quadrastichus erythrinae Kim. (Hymenoptera: Eulophidae).* Hawaii Department of Agriculture, New Pest Advisory, No. 05-03.

Messing, R. H., S. Noser, and J. Hunkeler. 2009. Using host plant relationships to determine the origin of the invasive Erythrina gall wasp, *Quadrastichus erythrinae* Kim (Hymenoptera: Eulophidae). Biol. Invasions 11: 2233–2241.

Rubinoff D, B.S. Holland, A.S. Shibata, R.H. Messing, and M.G. Wright. 2010. Rapid invasion despite lack of genetic variation in the Erythrina gall wasp (*Quadrastichus erythrinae* Kim). Pac. Sci. 64: 23–31.

# Classical biological control of cottony cushion scale with *Rodolia cardinalis*: towards ecosystem restoration in the Galápagos Islands

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The cottony cushion scale, *Icerya purchasi* Maskell (Hemiptera: Margodidae), a cosmopolitan sap sucking plant pest native to Australia was discovered in the Galápagos in 1982. It readily dispersed to at least 15 different islands in the Galápagos archipelago. *Icerya purchasi* has been recorded infesting 98 native or endemic plants, 17 of which are on the IUCN Red List of Threatened Species, of which a further five are classified as Endangered or Critically Endangered. In 1996, the Charles Darwin Foundation and the Galápagos National Park Service formed a Technical Advisory Committee to address the *Icerya* invasion. The Committee concluded that biological control with the predatory beetle, *Rodolia cardinalis* Mulsant (Coleoptera: Coccinellidae), offered the best prospect for permanent and widespread suppression of *Icerya*. In 1999, *Rodolia* was imported into Quarantine at the Charles Darwin Research Station (CDRS) to undergo rigorous safety testing to evaluate the threat this natural enemy would pose to non-target species, especially native or endemic insects. Analysis of quarantine studies coupled with the published studies on the use of *R. cardinalis* for *I. purchasi* control in numerous other countries led to the conclusion that this natural enemy would not present a significant threat to non-target species in the Galápagos (Causton et al. 2004, Lincango et al. 2011). In 2002, *R. cardinalis* was released onto 10 different islands in the Galapagos Archipelago. This was the first time that Galapagos authorities had approved the introduction of a biological control agent to these iconic islands.

Seven years after the release of *R. cardinalis*, the biological control program was considered mature enough to be evaluated vigorously in terms of successful suppression of the target pest, *I. purchasi*, and for non-target impacts. In October 2009, a two year evaluation project was initiated to assess the impact of *R. cardinalis* on *I. purchasi.* The project had three major objectives: 1) to survey islands for the presence of *I. purchasi* and *R. cardinalis* in urban, agricultural, and natural areas in Galápagos, 2) to measure the degree of suppression of *I. purchasi* by *R. cardinalis*, and 3) to investigate under field-like conditions the hypothesis that *R. cardinalis* has a limited prey range as predicted by quarantine laboratory studies.

***Monitoring Islands for the Presence of I. purchasi and R. cardinalis:*** Islas Santa Cruz[a,b], Baltra[a,b], Isabela[a,b], Floreana[a,b], San Cristóbal[a,b], Champion[a], Española[a], Marchena[b], and Fernadina[a,b] were surveyed for the presence of *I. purchasi* and *R. cardinalis* via visual observations of native plants ([a]performed for *I. purchasi* and *R. cardinalis*) or with sticky traps hung in bushes and trees ([b]to capture *R. cardinalis*). *Icerya purchasi* and *R. cardinalis* were found on all of the islands surveyed except Española. *Icerya purchasi* and *R. cardinalis* have not been reported previously from this island. *Rodolia cardinalis* was not released on either Baltra or Champion Islands in 2002 indicating that it has colonized these islands without human assistance.

***Measuring Suppression of Icerya Populations by R. cardinalis:*** To determine the degree of suppression of *I. purchasi* populations by *R. cardinalis* on several major native plants in relatively undisturbed native habitats four study sites were selected on Santa Cruz Island. These sites were monitored monthly and numbers of *I. purchasi* and *R. cardinalis* from timed searches of selected plants were recorded over a 2-yr period (October 2009 - November 2011). Survey results suggest that overall *I. purchasi* populations were low over the 2-yr period. For example, prior to the release of *R. cardinalis*, white mangroves, *Laguncularia racemosa* (L.) Gaertn. F. (Combretaceae), were heavily infested with *I. purchasi*. Following the release of *R. cardinalis* in 2002, *I. purchasi* populations declined on white mangroves (Calderón Alvarez et al. 2012). Between 2009-2011, *I. purchasi* populations remained very low on white mangroves at sites that were heavily infested prior to the commencement of the biological control program (Figure 1).

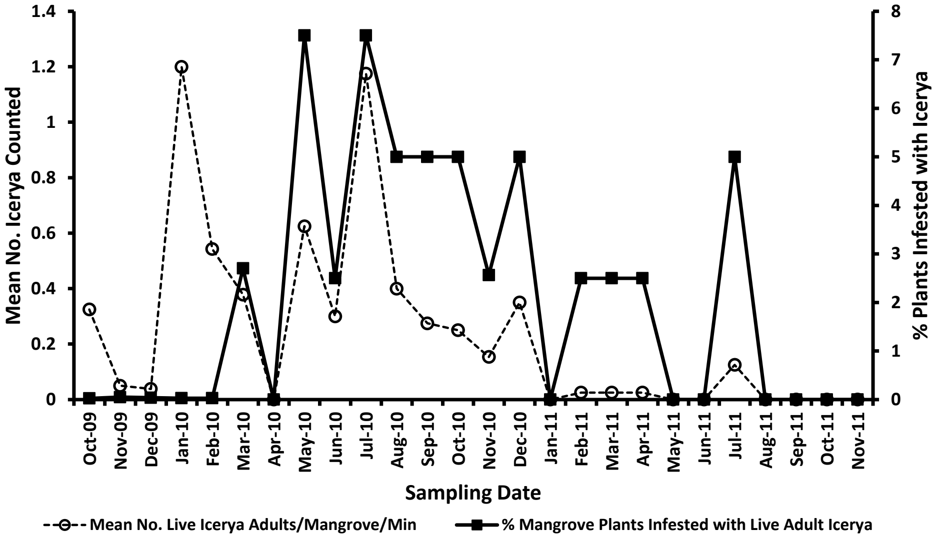

Figure 1. Monthly monitoring of *Icerya* populations on white mangroves at Playa Estación over a 2-yr period indicate that this pest was consistently maintained at very low levels (typically less than 1 adult *I. purchasi* per plant and less than 8% of sampled plants being infested.)

In some areas of Santa Cruz Island and on certain host plants, *I. purchasi* populations persisted at notably higher densities in comparison to other sites that were surveyed. The most notable of these sites was the *Icerya* infestation on *Scaevola plumieri* (L.) (Goodeniaceae) at Tortuga Beach. At this study site, *I. purchasi* was consistently present, but the percentage of infested plants increased and decreased over the 2-yr survey period. As pest densities increased, *R. cardinalis* populations rebounded and approximately 2-6 months later *I. purchasi* populations would decline again as predator populations peaked.

*Icerya purchasi* populations were monitored for 12 months on San Cristóbal Island at two different sites, an undisturbed natural area on Cerro Colorado dominated by *Waltheria ovata* Cav. (Sterculiaceae) and *Rhynchosia minima* (L.) DC (Fabaceae), and a mixed variety of plants (e.g. citrus, guava, *W. ovata* and *R. minima*) in a largely urbanized area. Similar population trends were observed on San Cristóbal as Santa Cruz, when *R. cardinalis* populations increased on infested plants, *I. purchasi* numbers declined. The presence of ants tending *I. purchasi* maybe disadvantageous for *R. cardinalis*. Field surveys on San Cristóbal indicated for both urban and wilderness areas, that ants were associated with high density populations of *I. purchasi*, and *R. cardinalis* presence was low or absent.

**Investigating the Prey-Range of *R. cardinalis* under Field-Like Conditions:** The feeding preferences and behavior of *R. cardinalis* were studied from October - December, 2009 in large cages at CDRS (Figure 2). The cages held native plants (e.g., *Parkinsonia aculeata* L. (Caesalpinaceae), *Gossypium* spp. (Malvaceae), *Acacia spp.* (Fabaceae), and *W. ovata*) that were infested with *Icerya* (the target) and non target prey species (e.g. *Coccus viridis* (Green), *Ceroplastes*, spp. (Hemiptera: Coccidae), mealybugs, aphids, and spider mites). Starved field collected and lab reared adult *R. cardinalis* were released into cages and their foraging behaviors and prey choices were recorded visually. More than 30 adult beetles were observed and almost 30 hours of visual observations were made. *Rodolia cardinalis* was never observed attacking non-target species. All feeding and oviposition events occurred on the target, *I. purchasi*. These results and our field observations support previous quarantine findings that showed that *R. cardinalis* is an extreme specialist on *I. purchasi* and it is very unlikely to feed on other insects in the Galápagos.

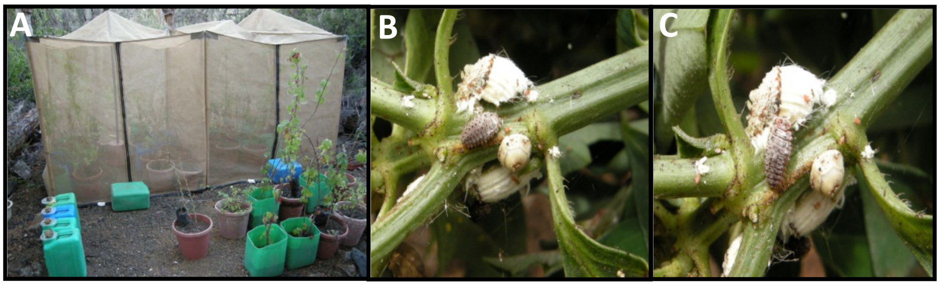

Figure 2. A. Field cages used for behavioral observations. B. *Rodolia* larva searching for prey. C. *Rodolia* larva ignores *Ceroplastes* and attacks Icerya.

Results from this two year project indicate: 1) the introduced predator *R. cardinalis* has survived and spread since its initial introduction into the islands in 2002, it is now widely present in many areas and habitats on at least six islands where it was released, and has managed to colonize at least two other islands infested with *I. purchasi* without human assistance, 2) *Icerya* has been suppressed to non-damaging levels on many important native host plants, including white mangrove, *Acacia* species, *W. ovata,* and *P. aculeata*, all of which are ecologically important species that were heavily attacked by *I. purchasi* before the release of *R. cardinalis* (Notable exceptions existed on certain host plants, especially *S. plumieri* and *R. minima*, or when honey-dew collecting ants were present and guarded *Icerya* from *Rodolia*), 3) there was no evidence of attack by free-ranging *R. cardinalis* on non-target insects from more than 30 h of behavioral observations in large field cages where a range of alternative prey species were provided on potted native plants.

From the results of two years of population monitoring of *I. purchasi* in several distinct habitats coupled with behavioral studies on the predation preferences of *R. cardinalis*, it is concluded that the biological control program has been very successful in two regards: 1) permanently suppressing damaging populations of the target pest, *I. purchasi*, on a wide variety of host plants in diverse habitats, and 2) safety, as no evidence for adverse non-target impacts has been found.

## References

Calderón Alvarez, C., C. E. Causton, M. S. Hoddle, C. D. Hoddle, R. G. Van Driesche, and E. J. Stanek. 2012. Monitoring the effects of *Rodolia cardinalis* on *Icerya purchasi* populations on the Galapagos Islands. BioControl 57: 167–179.

Causton C. E., M. P. Lincango, and T.G.A. Poulsom. 2004. Feeding Range Studies of *Rodolia cardinalis* (Mulsant), Candidate Biological Control Agent of *Icerya purchasi* Maskell in the Galápagos Islands. Biol. Control 29: 315–325.

Lincango, M., C. E. Causton, C. Calderón Alvarez, and G. Jiménez-Uzcátegui. 2011. Evaluating the safety of *Rodolia cardinalis* to two species of Galapagos finch; *Camarhynchus parvulus* and *Geospiza fuliginosa*. Biol. Control 56: 145–149.

# Update on conservation benefits from successful biological control of *Orthezia* scale on the island of St. Helena, South Atlantic Ocean

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The small South Atlantic island of St Helena has a highly degraded but internationally significant terrestrial flora, now covering only 1% of its land area (Cronk 1989). The 2500 gumwood trees, *Commidendrum robustum* DC (Asteraceae), in the last two natural stands, are an important part of this remnant flora. In 1991, a scale insect infesting the gumwoods was identified as *Orthezia insignis* (Hemiptera: Ortheziidae).

By 1993, there were severe infestations of the scale (Figure 1), and over 100 gumwood trees were dead. If the exponential increase in the number of dead trees had continued, all 2500 trees would have been killed by 1995.

Figure 1. *Orthezia insignis* infesting a gumwood shoot in 1993

Over 5000 *Hyperaspis pantherina* (Coleoptera: Coccinellidae) were released on St Helena from June 1993 to February 1994 (Figure 2).

Figure 2. Adult of *Hyperaspis pantherina* (length 2.5 mm) and dead *O. insignis*

Visual monitoring of gumwood shoots showed that *H. pantherina* numbers increased in 1994, coinciding with a 30× decrease in mean scale numbers. There was no further scale-induced tree mortality. Biological control of *O. insignis* was successful, but the extensive blackening from sooty moulds on all surviving gumwood trees in February 1995, suggested that the predator was effective only just in time to prevent most of the trees being killed. Experimental transfers of *O. insignis* showed that the other three members of the endemic genus *Commidendrum* were at risk from the scale. The introduction of *H. pantherina* into St Helena is an early example of biological control being initiated solely for conservation of indigenous biodiversity. It appears that this successful programme has saved the field population of a rare endemic plant from extinction (Fowler 2004).

In 2000 large scale restoration plantings of *C. robustum* began on St Helena (Figure 3). The aim was to establish a “Millenium Forest” covering 250ha of what was once part of the “Great Wood”, destroyed by human and feral animal use in the 19th century. To date 25ha has been planted and the community driven work continues with the project being awarded the prestigious Joint Nature Conservation Committee's Blue Turtle Award in 2010 for nature conservation in the UK Overseas Territories and Crown Dependencies.

Figure 3. Successful restoration planting of *C. robustum* in part of the Millennium Forest, St Helena.

This ambitious restoration project would have been impossible without successful control of *O. insignis*. The same argument can be made for the past success against *Icerya purchasi* Maskell (Hemiptera: Margodidae) using *Rodolia* *cardinalis* (Mulsant) (Coleoptera: Coccinellidae). Whilst there are problems with root and shoot mealybugs in the Millennium Forest, there are no reports of *O. insignis*. In the original stand of gumwoods at Peak Dale, which has been the main source of the seed for the new plantings, the only report of *O. insignis* is a brief outbreak in 2003 in unusually dry weather (St Helena Foundation 2012). The only other report of locally abundant *O. insignis* on the island was near to Jamestown where the scale was being attended by ants, and may have been protected from *H. pantherina*. Flow-on benefits to the highly endemic fauna on St Helena will have occurred from the successful biocontrol of scale insects because *Commidendrum* spp. support a range of host-specialist insects, particularly weevils (Basilewsky 1985).

## References

Basilewsky, P. 1985. The South Atlantic island of St Helena and the origins of its beetle fauna, pp. 257-275. *In* G.E. Ball (ed.), Taxonomy, phylogeny and zoogeography of beetles and ants. Dr. W. Junk Publishers, Dordrecht, The Netherlands.

Cronk, Q. C. B. 1989. The past and present vegetation of St Helena. J. Biogeogr. 16: 47–64.

Fowler, S. V. 2004. Biological control of an exotic scale, *Orthezia insignis* Browne (Homoptera: Ortheziidae), saves the endemic gumwood tree, *Commidendrum robustum* (Roxb.) DC. (Asteraceae) on the island of St. Helena. Biol. Control 29: 367-374.

St Helena Foundation. 2012. The Millennium Forest Project. http://www.sthelena.se/forest/intro.htm (accessed 24 July 2012).

# Effects of invasive scales on forest butterflies in Australia, and benefits following their biological control

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***Invasive scales and their effects on plants*.** Several species of exotic scale insects (Hemiptera: Coccidae and Diaspididae) became serious pests of horticulture in Australia in the late 1800s and early 1900s, gaining entry to the mainland and nearby islands on imported ornamentals plants or fruit crops. Some scales targeted for biological control were subsequently controlled by introducing natural enemies from overseas (Wilson 1960, Waterhouse and Sands 2001). The programmes aimed to control scales damaging horticultural crops (Smith et al. 1997), but other ecosystems also benefited from biological control of the scales, including indigenous forests.

Infestations of the soft scales (Coccidae) on plants, reduces the sap flow and plant vigour, nutrient translocation, emergent growth, flowering, fruiting and seed production. Heavy scale infestations may lead to death of leaves, stems and sometimes whole plants. Also, the honeydew excreted by scales promotes growth of sooty moulds, reducing photosynthesis and attracting ants. The wax scales (*Ceroplastes* spp.) and the honey dew supporting growth of sooty mould, have caused problems for hikers and passers by, including staining of clothing and producing allergic reactions. The impacts by wax scales on indigenous plants were most serious in lowland subtropical rainforests, rare plant communities classified in Australia as *Critically Endangered Ecosystems*.

*Species of wax scales.* Four polyphagous species of *Ceroplastes* spp. (Hemiptera: Coccidae) invaded many different ecosystem types in Australia prior to biological control programmes. These species, *Ceroplastes destructor* Newstead (from South Africa), *C. rubens* Maskell (from Africa), *C. sinensis* Del Guerico (from Argentina) and *C. ceriferus* Fabricius (from South America), reproduced parthenogenetically, with each scale producing up to 3,000 crawlers (Smith et al. 1997). Other soft scales of little concern in natural forests but important horticultural pests (Smith et al. 1997) include *Coccus hesperidum* (L.), *C. longulus* (Douglas), *C. viridus* (Green), *Saisettia oleae* (Oliver) and *S. coffeae* (Walker).

*Morphology. Ceroplastes destructor* characteristically produces moist and soft, white wax domes covering individual scales, or wax coalescing in masses and covering stems where the crawlers have settled in aggregations. The wax can completely cover leaf petioles, twigs and stems of the host plant (Snowball 1969). *Ceroplastes rubens* secretes firm pink wax domes from individuals on the leaves, or the wax can coalesce in heavy infestations on leaf midribs and twigs (Smith et al. 1997). Adult *C. sinensis* secrete pink and white wax, becoming grey when mature, as single domes or the groups of scales can form dense aggregations on stems and leaves. *Ceroplastes ceriferus* produces firm, cream wax domes with an anterior wax projection, with individual scales separated on twigs and stems of shrubs and on exposed roots of forest plants.

*Forest types infested*. *Ceroplastes destructor* became a very serious pest of cultivated citrus in eastern and south-western Australia in the late 1800s. Before the successful biological control projects in the 1960s and 1970s, *C. destructor* infested more than 100 species of plants in northern New South Wales (NSW) and south-eastern Queensland. Heavy infestations of the scale spread into open woodlands as well as shaded subtropical and tropical rainforests. For example, in Burleigh Heads National Park, Queensland, a wide range of rainforest plants became infested with *C.*

*destructor*, included in the families Araliaceae, Rutaceae, Pittosporaceae, Meliaceae, Rubiaceae, Sapindaceae and Myrtaceae (but not *Eucalyptus* spp.).

*Ceroplastes rubens* become a major pest on citrus and indigenous plants in mainland eastern Australian prior to the 1930s, afterwards spreading to Norfolk Island (Waterhouse and Sands 2001). Infestations developed mostly in sunlit woodlands and on rainforest regrowth of the same plant families affected by *C. destructor*, as well as Annonaceae. *Ceroplastes ceriferus* was of little concern in horticultural plantations but it spread into moist woodlands and rainforests in the 1950s, often attacking the stems and exposed roots of vines. Another wax scale, *Ceroplastes sinensis*, became established in eastern Australia in the 1960s (Snowball 1970), attacking citrus, exotic and indigenous trees, including *Ficus* spp. (Moraceae) and *Melaleuca* spp. (Myrtaceae) in parklands, gardens and in indigenous forests.

***Effects of scale infestations on insect host plants.*** *Ceroplastes destructor* attacking plants in many protected areas in north-eastern NSW and south-eastern Queensland, were monitored before and after the release of biological control agents, to provide information on the scale abundances, host plants, and other insects present including existing natural enemies (Sands et al. 1986). In rainforest, plants (100 m apart) were tagged and scale counts and ratings for infestation levels were recorded. Plants with butterfly larvae attended by ants were monitored and changes in their abundance recorded.

While indigenous insects appeared to avoid the scale-infested plants, in woodlands and rainforests, heavy infestations of *C. destructor* and *C. rubens* had impacts on butterflies by reducing flowing and the nectar available, particularly on *Pittosporum undulatum* Vent., *Auranticarpa rhombifolia* (A. Cunn.) ex Hook, *Hymenopsorum flavum* (Hook.) F. Muell. (Pittosporaceae) and *Burseria spinosa* Cav. (Burseraceae),. Abundant wax scales retarded leaf growth and production of flower buds (e.g. especially in Sapindaceae), as well as debilitating growth of other plant parts that were attractive to insects.

Many of the plants infested by wax scales were food plants for larvae of indigenous insects, including butterflies. In rainforest, *C. ceriferus* formed colonies on the rare vine *Pararistolocia praevenosa* (F. Muell.) Michael J Parsons (Aristolochiaceae), the principal food plant for the threatened subtropical Richmond Birdwing butterfly, *Ornithoptera richmondia* (Gray) (Sands 2008). *Ceroplastes ceriferus* weakened new growth and induced toughened of the leaves of the food plant, making leaves unsuitable for feeding by 1st instar larvae of the birdwing butterfly. *Ceroplastes ceriferus* also developed heavy infestations on *Dodonaea viscosa* Jacq. (Sapindaceae), a common woodland shrub that is a food plant for many herbivorous insects, including butterflies.

Impacts on herbivorous insects by *C. destructor* and *C. rubens*, occurred when the wax prevented access to plant parts for oviposition, when wax restricted movement between leaves, or the sooty mould and honeydew made foliage unsuitable for feeding. The movement of larvae from hollow stems or shelters to the foliage was particularly restricted by the aggregations of moist wax.

***Increase of exotic ants attracted to honeydew from scales.*** Leaves and shoots on scale-infested plants became contaminated by honeydew that supported growth of sooty mould, and the honeydew attracted high densities of several species of exotic ants, preventing indigenous ants from attending the larvae of lycaenid butterflies and Hemiptera. Exotic ants also interfered with natural enemies (e.g. lacewings and lady birds) of other insects, including scales (Snowball and Milne 1973), and these ants became predators of the larvae of butterflies. The stems of many rainforest mistletoes, e.g. *Lysiana subfalcata* (Hook.) Barlow (Loranthaceae), on *Capparis* spp.

(Capparaceae), became heavily infested by *C. destructor*, and ants attending the scales became predators of the immature stages of several species of butterflies (*Delias* sp., *Ogyris* ssp. and *Candalides margarita* [Semper]), feeding on the mistletoes.

Attracted by the honeydew from *C. destructor* in rainforest, the exotic ants *Paratrechina longipes* (Latreille), *Pheidole megacephala* (Fabricius), and *Tetramorium bicarinatum* (Nylander) (Hymenoptera: Formicidae), displaced indigenous ants (Burwell 2007) attending two species of butterfly larvae. In Burleigh Heads National Park, the rare rainforest butterflies, *Hypochrysops miskini* (Waterhouse) and *Pseudodipsas cephenes* Hewitson (Lepidoptera: Lycaenidae), declined in abundance when the exotic ant, *P. megacephala*, displaced *Anonychomyrma gilberti* (Shattuck) (Hymenoptera: Formicidae), the only ant attending larvae of the butterflies on the food plants. The exotic ants also displaced *A. gilberti* nesting in the hollow leaves and branches of trees.

In woodlands at Castle Hill, NSW, an indigenous ant, *Papyrius* sp. (*nitidus* group) was displaced by exotic ants attending *C. destructor*, particularly *Pheidole megacephala* (Fabricius) (Hymenoptera: Formicidae), occupying rocks and debris previously used as nest sites by the indigenous *Papyrius* sp., the only ant that tends larvae of the rare butterfly, *Hypochrysops ignitus* (Leach) (Lepidoptera: Lycaenidae). *Papyrius* sp. is also a host for other lycaenid butterflies (*Acrodipsas* spp.) whose predatory larvae live only in the nests of this ant. Until biological control of *C. destructor*, scale infested plants (e.g., *Dodonaea* spp.) that attracted exotic ants, were deserted as hosts by *H. ignitus* at Bobbin Head, NSW. The butterfly and its specific ant disappeared from many other well known breeding sites.

*Ceroplastes destructor* and exotic ants also caused declines in other woodland butterflies, for example at Castle Hill, NSW. The food plant (*B. spinosa*) of two common “copper” butterflies, *Paralucia pyrodiscus* (Doubleday) and *P. aurifer* (Blanchard) (Lepidoptera: Lycaenidae), became very heavily infested with *C. destructor* and, as the leaves of *B. spinosa* became increasingly blackened and the stems covered by wax. The two ants, *Notoncus capitalis* Forel and *Anonychomyrma* sp. [*nitidiceps* group] (Hymenoptera: Formicidae) tending the larvae of the copper butterflies, were displaced by exotic ants.

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***Biological control of scales**.* The four species of wax scales initially damaged forest plants and crops in coastal regions of Australia, but successful biological control programmes have reduced the significance of all scales previously considered pests of horticulture and natural ecosystems. *Ceroplastes destructor* was controlled by two South African parasitoids introduced into Australia from1968-1972 (Sands et al. 1986, Waterhouse and Sands 2001), *Anicetus communis* (Annecke), controlled the scale in temperate regions and *A. nyasicus* (Compere) (Hymenoptera: Encyrtidae) was the widely effective agent in tropical and subtropical regions. The significance of *C. rubens* as a pest in crops decreased after, *Anicetus beneficus* Ishii & Yasumatsu (Hymenoptera: Encyrtidae), was introduced from Japan in 1977 (Smith 1986), but occasional outbreaks of the scale have continued to occur in crops and on indigenous plants. In forests, *C. ceriferus* and *C. sinensis* eventually declined in abundance, from the transfer of attack by agents introduced to control other Coccidae and neither species is now abundant. However, occasional outbreaks of *C. sinensis* occur in NSW, mainly on exotic plants.

***Recovery of indigenous vegetation and insects**.* In the 1950s and early 1960s in Kuringai Chase National Park, NSW, infestations of *C. destructor* increased, and exotic ants attending the scales built up in abundance. By 1972 soon after establishment of the scale parasitoids, infestations of the scale were reduced to almost undetectable levels, and most butterflies began returning to previously known scale-infested sites. However, there were no signs of the immature stages of the rare butterflies, *H . miskini* and *P. cephenes* until 1978, when larvae of *H. miskini* were again seen being attended by the indigenous ant, *A. gilberti*. *Pseudodipsas cephenes* appeared to recover initially after control of the scale and the reduction in exotic ants, but this butterfly has not returned to normal numbers in other parts of south-eastern Queensland. Since biological control of the scales, the flowering plants, *Pittosporum undulatum, B. spinosa* and *H. flavum*, major sources of nectar for the butterflies, have recovered from scale infestations.

## References

Burwell, C. 2007. Ants of Brisbane. A Queensland Museum Wild Guide. Queensland Museum. 68 pp.

Sands, D. P. A. 2008. Conserving the Richmond Birdwing Butterfly over two decades: Where to next? Ecol. Manag. Rest. 9: 4–16.

Sands, D. P. A., Lukins, R. G and Snowball, G. J. 1986. Agents introduced into Australia for biological control of *Gascardia destructor* (Newstead: Coccidae). J Aust. Entomol. Soc. 25: 51–59.

Smith, D. 1986. Biological control for *Ceroplastes rubens* Maskell, by the introduced parasite *Anicetus beneficus* Ishii and Yasumatsu. Queensland J. Agric. Anim. Sci. 43: 101–105.

Smith, D, Beattie, G. A. C. and Broadley, R. (eds.) 1997. Citrus pests and their natural enemies. Integrated pest management in Australia. Queensland Department of Primary Industries, Information Series Q 1970, 272 p.

Snowball, G. F. 1969. Prospects for biological control of white wax scale. J. Entomol. Soc. Aust. (NSW) 5: 23–33.

Snowball, G. F. 1970. *Ceroplastes sinensis* Del Guercio (Homoptera: Coccidae), a wax scale new to Australia. J Aust. Entomol. Soc. 9: 57–64.

Snowball, G. F. and Milne, W. 1973. Protection by ants as a deterrent to biological control of scales. CSIRO Division of Entomology Annual Report 1972-1973.

Wilson, F. 1960. A review of the biological control of insects and weeds in Australia and Australian New Guinea. Technical Communication No. 1, Commonwealth Institute of Biological Control. Commonwealth Agricultural Bureau, London, UK, 102 pp.

Waterhouse, D. F. and Sands, D. P. A. 2001. Classical biological control of arthropods in Australia. ACIAR Monograph No. 77, 560 pp.

# Biological control of cycad scale, *Aulacaspis yasumatsui*, attacking Guam's endemic cycad, *Cycas micronesica*

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Despite attempted classical biological control with a predator and two parasitoids, greater than 90% of Guam's endemic *Cycas micronesica* K.D. Hill (Cycadaceae) plants have been killed since the island was invaded by the cycad aulacaspis scale (CAS), *Aulacaspis yasumatsui* Takagi (Hemiptera: Diaspididae), in 2003 (Marler and Lawrence, 2012). Prior to this invasion, *C. micronesica* was the most numerous plant species in Guam's forests with stem diameters greater than five inches (Donnegan et al. 2004). The CAS infestation was so severe that by 2006 *C. micronesica* was listed as endangered by the International Union for Conservation of Nature (Marler et al. 2006). This ecological disaster is still unfolding. Marler and Lawrence (2012) predict extirpation of wild cycads on Guam by 2019 if current trends persist.

*Aulacaspis yasumatsui*, described by Takagi (1977), is considered a minor pest of *Cycas* within its native Asian range (Anonymous 2006a), presumably as a result of regulation by natural biological control organisms. Outside of its native range, where CAS has escaped its natural enemies, it is a very serious pest of *Cycas* spp. This scale insect infests all parts of the plant including roots and reproductive structures. CAS is small enough to invade minute cracks and crevices where it is undetectable during quarantine inspections (Marler and Moore 2010). In the absence of chemical or biological control, infested plants become totally encrusted with multiple layers of CAS within a few months and die within a year (Anonymous 2006a). Accidental introduction of CAS to Florida in the 1990s (Howard et al. 1999) initiated subsequent invasions of the pest throughout several other states within the United States and other countries (Anonymous 2006b). In the Pacific, CAS was first detected in Hawaii in 1998, Taiwan in 2000, Guam in 2003, Rota in 2007, and Palau in 2008. The presumed pathway for this invasive species is movement of scales attached to cycads in the ornamental plant trade, although accidental, long-range movement of scale crawlers is an alternate invasion pathway.

*Aulacaspis yasumatsui* infestation on Guam progressed very rapidly. Initial detection in December, 2003 was on *Cycas revoluta* Thunb. (Cycadaceae) and *C. micronesica* growing in floral displays at the entrances to two of Guam's major hotels. Within a year the infestation had spread into a nearby population of wild *C. micronesica* and by 2006, the infestation was island-wide and plants had started dying in large numbers.

We observed no pre-existing natural enemies during frequent surveys of infested plants. A predator, *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae) and a parasitoid, *Coccobius fulvus* (Compere and Annecke) (Hymenoptera: Aphelinidae) were imported for CAS biological control during 2004 and 2005 (Moore et al. 2005). A second parasitoid, *Aphytis lignanensis* Compere (Hymenoptera: Aphelinidae), was imported in 2012. *Rhyzobius lophanthae* established rapidly. However, both parasitoids failed to establish in captivity and in the field.

About 100 *R. lophanthae*, were field collected on Maui, Hawaii in November 2004, flown to Guam and reared for one month in quarantine. Field releases on CAS-infested, wild *C. micronesica* at Ritidian Point were initiated in February 2005. The beetle established immediately and its initial population explosion peaked in the vicinity of the release site in June 2005, when we counted up to 57 adults per minute in visual inspections of infested wild *C. micronesica*. We also monitored adult beetles, scale crawlers, and male scales at Ritidian using yellow sticky cards arranged in a transect. The resulting time series data clearly indicate collapse of the CAS population following introduction of the predator followed by establishment of a dynamic equilibrium with scale levels near the trapping detection threshold (Figure 1). Following establishment at Ritidian, more than 7,450 laboratory-reared and field-collected *R. lophanthae* adults were introduced at 115 sites throughout Guam by collaborators.

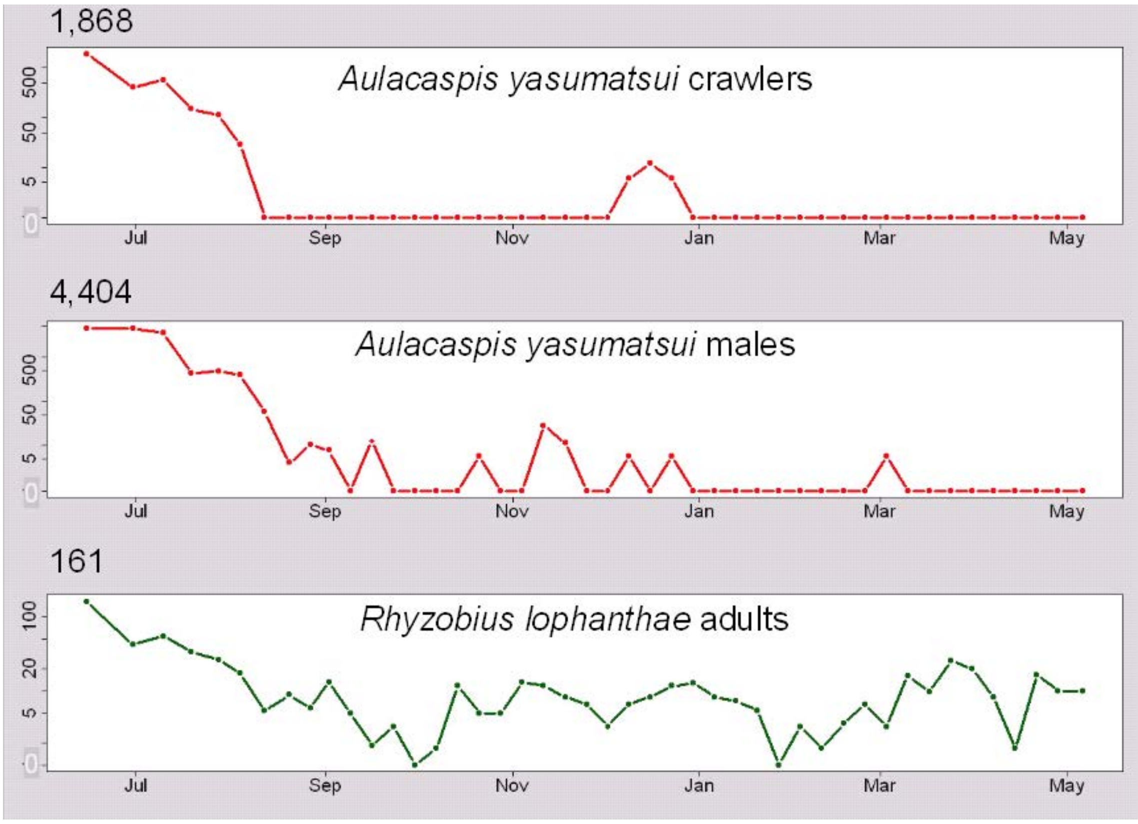

Figure 1. Insects trapped on yellow sticky cards at Ritidian Point, Guam following field release of *Rhyzobius lophanthae* in February, 2005. X axis runs from July 2005 through May 2006; Y-axis, in log scale, is number of insects trapped per square meter per day.

*Rhyzobius lophanthae* adults and grubs are voracious predators of CAS. Eggs are laid beneath female scale covers where 1st instar grubs consume the adult scale. Later instar grubs and adults feed on female and male scales. *Rhyzobius lophanthae* are currently ubiquitous throughout Guam. They are preventing mortality of mature cycads from scale infestation, but residual scales on these trees are preventing vigorous growth and seed production. More importantly, even though *R. lophanthae* are ubiquitous within their habitat, all *C. micronesica* seedlings become infested with CAS and eventually die (Marler and Lawrence 2012). Thus, with no reproduction occurring, health of the *C. micronesica* population is still in decline. We offer two explanations for the partial failure of *R. lophanthae* as a biological control agent for CAS:

1) Marler et al. (2013) provide evidence that the *R. lophanthae* predation rate decreases near the ground. This at least partially explains why seedlings are more vulnerable to mortality from scale infestation than mature plants.
2) *R. lophanthae* is much larger than CAS and it is not able to prey on individuals living in small cracks and crevices on the plant. CAS living in refugia provide a

> steady stream of crawlers which rapidly repopulate external surfaces of the plant during periods of low predation.

We suggest that there is a urgent need to introduce one or more smaller-sized biological control agents which are active near the ground and can follow CAS into its refugia.

Unfortunately, attempts to introduce CAS parasitoids to Guam have failed. A Chinese strain of *Coccobius fulvus* from Florida was imported and released several times starting in 2005. On each occasion, the parasitoids died out both in the field and the laboratory, probably out-competed by *R. lophanthae* (G.V.B. Reddy, personal communication). We are currently attempting to introduce *A. lignanensis* which coexists with *R. lophanthae* as a CAS biological control agent in Texas (Flores and Carlson 2009) and Hawaii (B. Kumashiro, personal communication). In 2012, we imported about 100 *A. lignanensis* adults from Honolulu, Hawaii. These wasps were reared from CAS infesting *C. revoluta* in a home garden (There are no wild cycads in Hawaii). We placed these wasps in a cage containing CAS-infested *C. micronesica* leaves. We had carefully removed all visible *R. lophanthae* adults and grubs from these leaves, but there were enough beetle eggs and 1st instar larvae hiding beneath scale covers to consume all scales before any adult wasps emerged. In our next attempt, we will present imported *A. lignanensis* with caged *C. revoluta* infested with CAS but without *R. lophanthae*.

Our immediate objective is to establish a biological control agent, in addition to *R. lophanthae*, which will provide adequately protect *C. micronesica* seedlings from CAS-related mortality so that this important endemic plant species can start to recover.

## References

Anonymous 2006a. Pest alert: Cycad aulacaspis scale. International Union for Conservation of Nature, Species Survival Commission, Cycad Specialist Group. URL http://www.cycadsg.org/publications/CAS/Cycad-Aulacaspis-Scale-Pest-Alert.pdf.

Anonymous. 2006b. Report and recommendations on cycad aulacaspis scale, *Aulacaspis yasumatsui* Takagi (Hemiptera: Diaspididae). International Union for Conservation of Nature Species Survival Commission, Cycad Specialist Group, Subgroup on Invasive Pests. URL http://www.cycadsg.org/publications/CAS/CSG-Report-on-Cycad-Aulacaspis-Scale.pdf.

Donnegan, J. A., S. L. Butler, W. Grabowiecki, B. A. Hiserote, and D. Limtiaco 2004.Guam's forest resources, 2002. USDA Forest Service, Pacific Northwest Research Station, Portland OR. URL http://www.fs.fed.us/pnw/pubs/pnw_rb243.pdf

Flores, D., and J. Carlson 2009. Fortuitous establishment of *Rhyzobius lophanthae* (Coleoptera: Encyrtidae) in South Texas on the cycad aulacaspis scale, *Aulacaspis yasumatsui* (Hemiptera: Diaspididae). Southwest. Entomol. 34: 489–492. URL: http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1079&context=entomologyother.

Howard, F. W., A. Hamon, M. McLaughlin, T. Weissling, and S. L. Yang 1999. *Aulacaspis yasumatsui* (Hemiptera: Sternorrhynchidae: Diaspididae), a scale insect pest of cycads recently introduced into Florida. Fla. Entomol. 82:1427, 1999. URL http://journals.fcla.edu/flaent/article/view/74858.

Marler, T. E., J. Haynes, and A. Lindstrom 2006. Cycas micronesica. IUCN Red List of Threatened Species. URL http://www.iucnredlist.org/details/61316/0.

Marler, T. E., and J. H. Lawrence 2012. Demography of *Cycas micronesica* on Guam following introduction of the armoured scale *Aulacaspis yasumatsui*. J. Trop. Ecol. 28:233–242. URL http://www.wptrc.org/userfiles/file/Marler/Cycas%20survival%20correction.pdf.

Marler, T. E., and A. Moore 2010. Cryptic scale infestations on *Cycas revoluta* facilitate scale invasions. HortScience 45:837–839. URL http://www.wptrc.org/userfiles/file/Marler/CAS%20refugia%20reprint.pdf.

Marler, T. E., R. H. Miller, and A. Moore. 2013. Vertical stratification of predation on *Aulacaspis yasumatsui* infesting *Cycas micronesica* seedlings. HortScience 48(1): 1-3.

Moore, A., T. E. Marler, R. H. Miller, and R. Muniappan 2005. Biological control of cycad aulacaspis scale on Guam. Cycad Newsletter 28(5) 6-8. URL http://www.cycadsg.org/publications/CAS/TCS-Moore-etal.pdf

Takagi, S. 1977. A new species of *Aulacaspis* associated with a cycad in Thailand (Homoptera: Coccoidea). Insecta Matsumur., New Series 11: 63–72.

# Biological control and biological conservation – prospects for a happy marriage

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Historically, conservation biologists and conservation-oriented invasion biologists have been wary of classical biological control, largely because of such prominent early misguided projects as those that spread the small Indian mongoose to islands worldwide for rat control, brought the cane toad to Australia to control beetles, and introduced the rosy wolf snail to Pacific islands to combat the giant African snail (Simberloff 2012). In each of those cases the biological control agent threatened or even caused the extinction of several species of conservation concern. Nevertheless, biological control successes in agriculture and rangeland are also well known and cannot but help to intrigue conservationists, a process additionally spurred by the advocacy of respected biological control professionals of widespread use of biological control for conservation purposes (e.g., Van Driesche et al. 2010). What are the prospects that this matchmaking will succeed?

Foremost among conservationists' concerns is the possibility of direct non-target impacts on species of concern. To a great extent the threat of an immediate impact of this sort can be minimized for insects introduced to control pest plants by rigorous use of expanded versions of the centrifugal phylogeny method (Wapshere 1974), including no-choice tests on a large sample of native plants (Simberloff 2012). For entomophagous biological control agents, it will be more difficult to allay conservationists' concerns, because there is usually no currently feasible version of the centrifugal phylogeny method (Messing 2001), and because phylogeny of potential target species is not as reliable an indication of actual use as for plants.

The spread of the cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), from the Lesser Antilles, where it was introduced to control pest cactus,*Opuntia* spp. (Cactaceae) to Florida and beyond, where it threatens native cacti of conservation concern (Zimmermann et al. 2000), is well-known among conservationists. So is the unauthorized deliberate spread of the salt cedar beetle, *Diorhabda carinulata* Desbrochers (Coleoptera: Chrysomelidae), beyond its intended area of use (Dark 2009). These cases contribute to conservationists' unease about biological control introductions by raising the spectre that they will affect non-target species in areas where their use was unintended. This concern can never be totally allayed, but it can be minimized by being frank about the possibility of spread and by applying environmental niche modeling methods to attempt to account for possible range shift with climate change (Simberloff 2012).

A major thrust of ecology, invasion biology, and conservation biology research of the last two decades is focus on entire ecosystems rather than simply on species populations. This focus has revealed, among other things, a wealth of indirect effects of species on one another (e.g. apparent competition, trophic cascades) that have not typically been considered in decisions about whether to introduce biological control agents (Simberloff 2012). For instance, the centrifugal phylogeny method is all about direct impacts on species. If biological control is to be employed in the service of conservation, the possibility of indirect effects will have to be a more prominent part of decision-making. Yet such effects are notoriously difficult to predict.

Another outcome of ecosystem-oriented research is the recognition that many ecosystems have intricate, critical linkages – both trophic and otherwise – between pairs of species that would not, at a casual glance, appear to be interacting. In agriculture and rangelands with relatively simplified communities that are designed to produce specific products, the linkages are probably on average not nearly so complex and, in any event, it would not be considered so problematic if a critical linkage were sundered. However, in a natural ecosystem whose existence and normal functioning are desired for their own sakes (and possibly for multiple ecosystem services), severing or severely reducing the strength of a linkage could be a far more serious matter. If biological control (or any management method) is aimed at reducing a longstanding resident of an ecosystem, careful consideration will have to be given to the many possible linkages this target species may have formed with native species. A newly invading species, however, will not have had time to integrate itself into the resident community in such a way that its removal would have harmful unintended consequences (Simberloff et al. 2013), and biological control (indeed, any management method) in such cases would not raise this issue.

In the final analysis, perhaps the factor that would most boost the enthusiasm and optimism of conservationists about the usefulness or biological control in the conservation arena would be striking success in reducing the impact of one of the insect invasions currently threatening widespread, important, highly publicized forest ecosystems – the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), or the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae) come to mind. Biological control releases against the first two targets have been conducted with much hoopla, but the invasions continue to spread quickly. With visible success, concerns about the adequacy of tests on non-target insects (Simberloff 2012) would probably be swept aside in favor of the perceived greater good of saving an entire prized ecosystem. Local or regional biological control successes against aquatic plant invaders with conservation consequences (e.g., alligator weed, *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae), giant *Salvinia*, *S. molesta* D.S. Mitch. (Salvinaceae), and water hyacinth, *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae) are substantial but not too well known to the public, except locally. A major success against a devastating forest invader would be far more visible and would probably convert many conservationists from wary skepticism to thoughtful consideration, if not to outright enthusiasm.

## References

Dark, S. 2009. Beetles attack! City Weekly (Salt Lake City, Utah), March 11. http://www.cityweekly.net/utah/article-7512-beetles-attack.html (accessed 21. Dec. 2010).

Messing, R. H. 2001. Centrifugal phylogeny as a basis for non-target host testing in biological control: is it relevant for parasitoids? Phytoparasitica 29: 187–189.

Simberloff, D. 2012. Risks of biological control for conservation purposes. BioControl 57: 263–276.

Simberloff, D. S., J.-L. Martin, P. Genovesi, V. Maris, D. A. Wardle, J. Aronson, F. Courchamp, B. Galil, E. García-Berthou, M. Pascal, P. Pyšek, R. Sousa, E. Tabacchi, and M. Vilà. 2012. Impacts of biological invasions" what's what and the way forward. Trends in Ecology and Evolution 28: 58–66.

Van Driesche, R. G., R. I. Carruthers, T. Center, M. S. Hoddle, J. Hiough-Goldstein, L. Morin, L. Smith, D. L. Wagner, B. Blossey, V. Brancatini, R. Casagrande, C.E. Causton, J. A. Coetzee, J. Cuda, J. Ding, S. V. Fowler, J. H. Frank, R. Fuester, J. Goolsby, M. Grodowitz, T. A. Heard, M. P. Hill, J. H. Hoffmann, J. Huber, M. Julien, M. T. K. Kairo, M. Kenis, P. Mason, J. Medal, R. Messing, R. Miller, A. Moore, P. Neuenschwander, R. Newman, H. Norambuena, W. A. Palmer, R. Pemberton, A. Perez Panduro, P. D. Pratt, M. Rayamajhi, S. Salom, D. Sands, S. Schooler, M.

Schwarzländer, A. Sheppard, R. Shaw, P. W. Tipping, and R. D. van Klinken. 2010. Classical biological control for the protection of natural ecosystems. Biol. Control 54: S2–S33.

Wapshere, A. J. 1974. A strategy for evaluating the safety of organisms for biological weed control. Ann. App. Biol. 77: 201–211.

Zimmermann, H. G., V. C. Moran, and J. H. Hoffmann. 2000. The renowned cactus moth, *Cactoblastic cactorum*: its natural history and threat to native *Opuntia* in Mexico and the United States of America. Divers. Distrib. 6: 259–269.

# Classical biological control of the emerald ash borer and its potential for preserving ash-dependent biodiversity

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The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) (EAB), an Asian buprestid beetle, has currently spread to 18 States in USA and two provinces in Canada since it was first detected in 2002 in Michigan. It has since caused large-scale decline and mortality of North American ash, *Fraxinus* spp. (Olecaceae), trees, which are widely distributed in a diverse range of ecosystems, from dry, upland forests to swamps (MacFarlane and Meyer, 2005). The beetle also threatens rare ash species such as pumpkin ash, *Fraxinus profunda* (Bush) Bush, (Bauer, personal communication) as well as native arthropods that feed exclusively on ash (Gandhi and Herms 2010, Wagner 2007). A recent literature survey showed that 43 native arthropod species including 22 species of moth are exclusively associated with ash trees for either feeding or breeding purposes and thus face a risk of endangerment (Gandhi and Herms 2010, Wagner 2007). Extirpation of arthropods exclusively dependent upon ash may result in cascading effects on other affiliated species with which they may be inextricably connected. Therefore, failure in successful control or management of EAB spread and invasion in North America could have severe negative ecological impacts on biodiversity that exclusively dependent on ash trees.

Current control or management strategies for EAB involve the use of integrated approaches to: 1) reduce EAB populations in infested areas; and 2) slow the spread of EAB to the non-infested areas (Poland et al. 2010, Mercader et al. 2011). These approaches include delimitation of infested areas, regulatory restriction of movement of EAB-infested wood or plant materials, insecticide treatment or physical destruction of infested trees (including artificially girdled EAB trap trees), and biological control via release of natural enemies collected from EAB's native range (e.g. Liu et al. 2003, 2007, USDA APHIS 2007, McCullough et al. 2009, Poland et al. 2010, Mercader et al. 2011). Although none of these approaches alone appear to be sufficiently effective in containing the spread of EAB, biological control via self-propagating and dispersing natural enemies holds promise in reducing EAB populations, particularly in forested ecosystems.

A classical biological control program was initiated by the US Department of Agriculture shortly after the discovery of EAB in Michigan, and led to the introduction of three parasitoids from China in 2007: *Spathius agrili* Yang (Hymenoptera: Braconidae), *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), and *Oobius agrili* Zhang et al. (Hymenoptera: Encyrtidae) (Liu et al. 2003, 2007, USDA APHIS 2007). While the former two parasitoid species attack EAB larvae, *O. agrili* parasitizes EAB eggs. With the establishment of the USDA APHIS EAB biological control rearing facility in Brighton, Michigan in 2009, large numbers of the introduced parasitoids have since been released in many ash-dominated forest sites in 12 EAB-infested states. As of the fall 2011, field studies confirmed that one or more of these introduced parasitoids are successfully established in five states (Michigan, Maryland, Ohio, Illinois, and Indiana), although their combined impacts on EAB population growth or ash health are still not known (Bauer et al. 2008, 2009, 2010, Duan et al. 2010a and b, 2012, Gould et al. 2011). Populations of these exotic parasitoids should continue to disperse into more EAB-infested areas, increase over time, and exert significant control of EAB populations within next few years in the USA.

While the introduction and establishment of parasitoids from the native range of EAB (China and other parts of Northeast Asia) will likely continue to be a critical component of the EAB management strategies in North America, the benefit of such a classical biological control program is also likely to extend to other native arthropods that depend on ash resources. The presented research shows not only the challenge and potential of the classical EAB biological control program, but also its potential in preserving the ash-dependent biodiversity in North America.

**References**

Bauer, L. S., H. P. Liu, and D. L. Miller. 2009. Emerald ash borer biological control: rearing, releasing, establishment, and efficacy of parasitoids, pp. 7–8. *In* K. McManus, and K. Gottschalk (eds.), Proceedings 20th U. S. Department of Agriculture Interagency Research Forum on Invasive Species, 2009 January 13-16, Annapolis, MD. USDA Forest Service, Gen. Tech. Rep. NRS-P-51.

Bauer, L. S., H.P. Liu, D.L. Miller, and J. Gould. 2008. Developing a biological control program for emerald ash borer (*Agrilus planipennis*), an invasive ash pest in North America. Mich. Entomol. Soc. News. 53: 38–39. http://www.nrs.fs.fed.us/pubs/jrnl/2008/nrs_2008_bauer_002.pdf.

Bauer, L. S., J. Gould, and J. J. Duan. 2010. Can biological control of emerald ash borer save our ash? Mich. Entomol. Soc. News. 55: 26–27.

Duan, J. J., L. S. Bauer, M. D. Ulyshen, J. Gould, J., and R. Van Driesche. 2010a. Development of methods for the field evaluation of *Oobius agrili* (Hymenoptera: Encyrtidae) in North America, a newly introduced egg parasitoid of the emerald ash borer (Coleoptera: Buprestidae). Biol. Control 56, 170–174.

Duan, J. J., M. D. Ulyshen, L. S. Bauer, J. Gould, and R. Van Driesche. 2010b. Measuring the impact of biotic factors on populations of immature emerald ash borer (Coleoptera: Buprestidae). Environ. Entomol. 39: 1513–1522.

Duan, J. J., L. S. Bauer, K. J. Abell, and R. Van Driesche, R., 2012. Population responses of hymenopteran parasitoids to the emerald ash borer (Coleoptera: Buprestidae) in recently invaded areas in north central United States. BioControl 57: 199–209.

Gould, J., L. S. Bauer, J. J. Duan, I. Fraser, and M. Ulyshen, M. 2011. Recovery of parasitoids of the emerald ash borer, Agrilus planipennise, in Michigan, Ohio and Maryland. pp 24 –25. *In* K. McManus, and K. Gottschalk (eds.), Proceedings 22th U. S. Department of Agriculture Interagency Research Forum on Invasive Species, 2011 January 12-15, Annapolis, MD. USDA Forest Service Gen. Tech. Rep. NRS-P-92.

Gandhi K. J. K., and D. A. Herms. 2010. North American arthropods at risk due to widespread *Fraxinus* mortality caused by the Alien Emerald ash borer. Biol. Invasions 12: 1839–1846.

Liu, H. P., L. S. Bauer, R. T. Gao, T. H. Zhao, T. R. Petrice, R. A., and R. A. Haack. 2003. Exploratory survey for the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), and its natural enemies in China. Great Lakes Entomol. 36: 191–204.

Liu, H. P., L. S. Bauer, D. L. Miller, T. H. Zhao, R. T. Gao, L. Song, Q. Luan, R. Jin, R., and C. Gao. 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biol. Control 42: 61–71.

MacFarlane, D. W., and S. P. Meyer. 2005. Characteristics and distribution of potential ash tree hosts for emerald ash borer. Forest Ecol. Manag. 213: 15–24.

McCullough, D. G., N. W. Siegert, and J. Bedford. 2009. Slowing ash mortality: a potential strategy to slam emerald ash borer in outlier sites. *In* K. McManusand K. Gottschalk (eds.) Proceedings 20th U. S. Department of Agriculture Interagency

Research Forum on Invasive Species, 2009 January 13-16, Annapolis, MD. Gen. Tech. Rep. NRS-P-51. New Town Square, PA: USDA Forest Service Northern Research Station, pp. 44–46.

Mercader, R. J., N. W. Siegert, A. M. Liebhold, and D. G. McCullough. 2011b. Simulating the effectiveness of three potential management options to slow the spread of emerald ash borer (*Agrilus planipennis*) populations in localized outlier sites. Can. J. Forest Res. 41: 254–264.

Poland, T. M., D. G. McCullough, D. A. Herms, L. S. Bauer, J. R. Gould, and A. R. Tluczek. 2010. Management Tactics for emerald ash borer: chemical and biological control. *In* K. McManus and K. Gottschalk (eds.) Proceedings 21th U. S. Department of Agriculture Interagency Research Forum on Invasive Species, 2010 January 12-15, Annapolis, MD. Gen. Tech. Rep. NRS-P-75. New Town Square, PA: USDA Forest Service Northern Research Station, pp. 46–49.

Rice, K. B., and W. Klooster. 2010. Emerald Ash Borer Invasion of North American Forests. www.emeraldashborer.info.

USDA APHIS. 2007**.** The proposed release of three parasitoids for the biological control of the emerald ash borer (*Agrilus planipennis*) in the continental United States: environmental assessment. Federal Register 72: 28947–28948, Docket No. APHIS-2007-0060.

Wagner D. L. 2007. Emerald ash borer threatens ash-feeding Lepidoptera. N. Lepidop. Soc. 49: 10–11.

Yang, Z-Q., J. S. Strazanac, P. M. Marsh, C. Van Achterberg, and W-Y. Choi. 2005. First recorded parasitoid from China of *Agrilus planipennis*: A new species of *Spathius* (Hymenoptera: Braconidae: Doryctinae). Ann. Entomol. Soc. Amer. 98: 636–642.

Yang, Z-Q., J. S. Srazanac, Y-X. Yao, X-Y Wang. 2006. A new species of emerald ash borer parasitoid from China belonging to the genus *Tetrastichus* Haliday (Hymenoptera: Eulophidae). P. Entomol. Soc. Wash. 108: 550–558.

Zhang Y. Z., D. W. Huang, D. W. Uang, T. H. Zhao, H. P. Liu, and L. S. Bauer. 2005. Two new species of egg parasitoids (Hymenoptera: Encyrtidae) of wood-boring beetle pests from China. Phytoparasitica 33: 253–260.

# Winter moth: biological control and population dynamics in the northeastern United States

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The winter moth, *Operophtera brumata* L. (Lepidoptera: Geometridae), a leaf-feeding native of Europe, has recently invaded the northeastern United States and is causing widespread defoliation (Elkinton et al. 2010). Previous invasions by this species in Nova Scotia (Figure 1.) and British Columbia have been suppressed by the introduction of two parasitoids from Europe, *Cyzenis albicans* (Fallén) (Diptera: Tachinidae) and *Agrypon flaveolatum* (Gravenhorst) (Hymenoptera: Ichneumonidae) (Embree 1965, Roland and Embree 1995). As a result of these introductions, low-density populations of winter moth now persist indefinitely in these regions similar to those that exist in most parts of Europe. Between 2005 and 2011 we have introduced *C. albicans* at 15 locations in Massachusetts and Rhode Island. We focused on *C. albicans* and not *A. flaveolatum* because the latter species caused generally lower parasitism of winter moth in Canada and also it is polypahagous, whereas *C. albicans* is nearly monophagous and thus has much lower potential for attacking non-target species. Our first *C. albicans* recoveries occurred in 2010 at four of the six sites where we had released prior to that year. In 2012, we have the first evidence at one of these sites of significant levels of parasitism by *C. albicans* (approximately 30%). At these levels it should start to have a measurable impact on winter moth densities. We have now recovered *C. albicans* at all six of the earlier release sites, and at three of them we have noted increasing parasitism by this species.

In Nova Scotia in the 1950s, *C. albicans* was first recovered in 1959, five years after first release, but then it quickly suppressed winter moth permanently beginning in 1961 (Figure 1., Embree 1965, Roland and Embree 1995). We now expect that *C. albicans* parasitism and impact will follow a similar trajectory in New England. In both examples, there was delay of five or more years between first release and *C. albicans* recoveries followed by onset of significant levels of parasitism. The delay was very likely due to the fact that both host and parasitoid have but one generation per year, and in each case, only a few hundred or a few thousand *C. albicans* individuals were released into host populations that can number 100,000 larvae per tree or 20 million per ha.

We have now collected data on density and survival of different winter moth life stages at long-term population monitoring sites in eastern Massachusetts for the past nine years. We document two periods of high density (2004–2005 and 2009–2010). In both 2005 and 2010, there was a dramatic decline in larval densities. Preliminary analyses suggest that we have density-dependent mortality occurring in all life stages, as well as density-related changes in fecundity. We hope to assemble these findings into a comprehensive account of the dynamics of outbreak populations of winter moth prior to the establishment of *C. albicans* in New England. We will follow this with further studies of the impact of *C. albicans* on winter moth as it takes hold in each of these populations.

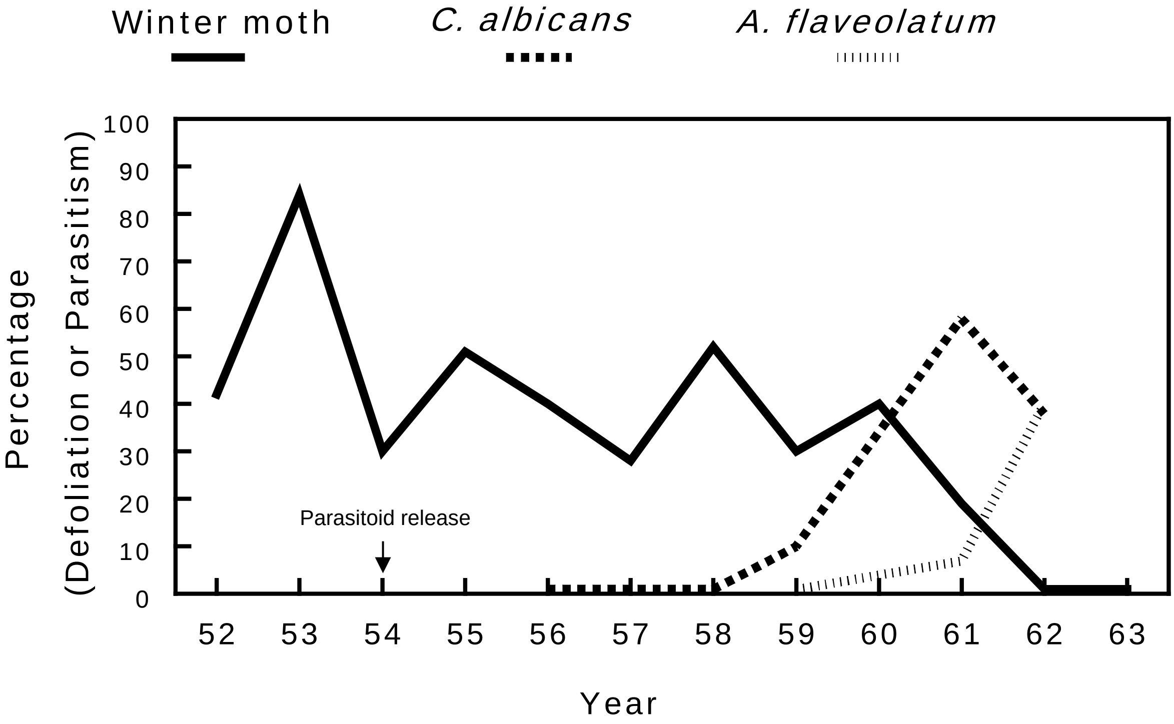

Figure 1. Percent defoliation by winter moth and percent parasitism by *Cyzenis albicans* and *Agrypon flaveolatum* at the Oak Hill release site in Nova Scotia 1952 - 1963. Figure redrawn from Embree, D. G. (1965).

## References

Elkinton, J. S., G. H. Boettner, M. Sremac, R. Gwiazowski, R. Hunkins, J. Callahan, S. B. Schuefele, C. Donahue, A. H. Porter, A. Khrimian, B. M. Whited, and N. K. Campbell. 2010. Survey for winter moth, (Lepidoptera: Geometridae) in northeastern North America with pheromone-baited traps and hybridization with the native Bruce spanworm. Ann. Entomol. Soc. Amer. 103: 135-145.

Embree, D. G. 1965. The population dynamics of the winter moth in Nova Scotia, 1954-1962. Mem. Entomol. Soc. Can. 46: 1–57.

Roland, J. and Embree, D. G. 1995. Biological control of the winter moth. Ann. Rev. Entomol. 40: 475–492.

# Practical steps toward enhancing cooperation between conservation biologists and biological control scientists

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Before the seminal article by Howarth (1991), classical biological control was seen as a wholly green technology by entomologists and conservation biologists alike, as the concern of the day was to reduce the clear damage to wildlife from widespread use of agricultural pesticides. However, this article laid out the case that unlike temporary damage from chemical pollutants, biological invaders were typically permanent in their effects. Howarth (1991) pointed out a number of cases in which it was known or believed that classical biological control introductions had reduced populations of native insects or plants. This stimulated two decades in which this view was novel enough to attract significant funding. During this period, many studies were done on particular cases to gather further insight into impacts on particular species (e.g. Boettner et al. 2000, Henneman and Memmot 2001, Stiling 2002, Louda et al. 2005, Johnson et al. 2005, Barratt et al. 2007, Finlayson et al. 2008, Kaufman and Wright 2009). While some studies clearly identified and quantified important damage to non-target species (Boettner et al. 2000, Stilling 2002, Louda et al. 2005, Barratt et al. 2007), others found that initial assumptions of impact were unfounded (Johnson et al. 2005, Kaufman and Wright 2009). Emergence of a more detailed understanding of the likelihood of risk to non-target species from classical biological control introductions emerged gradually (Simberloff and Stiling 1996, Lynch and Thomas 2000, Pemberton 2000, Louda et al. 2003, van Lenteren et al. 2006).

Broadly, risk of non-target impact of biological control agents as seen in the historical record is inverse to the year of agent introduction, with modern practice being safer than early introductions (Lynch and Thomas, 2000, Pemberton 2000). Also, the benefit of many biological control projects targeted at natural system invaders damaging to native biodiversity is widespread and substantial (Van Driesche et al., 2010). Therefore we are now at a more nuanced point in the discussion between biological control scientists and conservation biologists, a point in which mutual efforts to use biological control to protect biodiversity in natural ecosystems is feasible, if not yet widespread.

Methods therefore to encourage these two groups to work more closely together and have better mutual understanding are urgently needed. Here we discuss some ideas that may be helpful to move in that direction:

***Attend each other's meetings***. Working together means not working in isolation. One method to bring biological control scientists closer to conservation biology and invasion biology is for biological control scientists to attend meetings of conservation and invasion biologists. Hearing their concerns and issues in person will change minds. The converse also holds true: conservation biologists should be part of biological control meetings, not as "Davids in the lion's den", but as colleagues.

***Talk to each other*****.** Defending positions leads nowhere other than where you already are. Open up to hearing new things and talk to your colleagues in conservation biology (or from the reverse perspective, people from biological control) and let facts in particular cases, good or bad, form the basis of a better understanding on both sides.

***Develop more public tools for discussing host range estimation of new agents during the testing phase.*** The timing of exchange of information is critical. Biological control projects need conservation biologists' perspective and facts early in projects when actions are being taken, not late in projects after choices have already been made. To facilitate this early discussion, biological control scientists need a web-based forum to post plans for host range testing and creation of new biological control projects that will allow significant and early input from the conservation biology and invasion communities.

***Plan and carry out projects together***. Based on the above getting to know you steps, working friendships should emerge. Future biological control projects should be collaborations between conservation biologists and biological control scientists from the very beginning. Conservation biologists should be part of the system for making decisions as projects develop and should be active, "in-the-field" participants, seeing field conditions and events in person.

***And more***. Finally, this is not a defined process and new ideas certainly will emerge as the above developments take hold. Such new ideas, processes, techniques should be embraced as they occur.

## References

Barratt, B. I. P., C. M. Ferguson, A. S. Bixley, K. E. Crook, D. M. Barton, and P. D. Johnstone. 2007. Field parasitism of nontarget weevil species (Coleoptera: Curculionidae) by the introduced biological control agent *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) over an altitude gradient. Environ. Entomol. 36: 826–839.

Boettner, G.H., J. S. Elkinton, and C. J. Boettner. 2000. Effects of a biological control introduction on three nontarget native species of saturniid moths. Conserv. Biol. 14: 1798–1806.

Finlayson, C. J., K. M. Landry, and A. V. Alyokhin. 2008. Abundance of native and non-native lady beetles (Coleoptera: Coccinellidae) in different habitats in Maine. Ann. Entomol. Soc. Amer. 101: 1078–1087.

Henneman, M.L., and J. Memmott. 2001. Infiltration of a Hawaiian community by introduced biological control agents. Science 293: 1314–1316.

Howarth, F.G. 1991. Environmental impacts of classical biological control. Ann. Rev. Entomol. 36: 485–509.

Johnson, M. T., P. A. Follett, A. D. Taylor, and V. P. Jones. 2005. Impacts of biological control and invasive species on a non-target native Hawaiian insect. Oecologia 142: 529–540.

Kaufman, L. V., and M. G. Wright. 2009. Life history, seasonal phenology, and parasitoids of the Hawaiian endemic moth *Udea stellata* (Lepidoptera: Crambidae). Ann. Entomol. Soc. Amer. 102: 104–111.

Louda, S. A., R. W. Pemberton, M. T. Johnson, and P. A. Follett. 2003. Nontarget effects—the Achilles heel of biological control? Retrospective analysis to reduce risk associated with biocontrol introductions. Ann. Rev. Entomol. 48: 365–396.

Louda, S. M. , T. A. Rand, A. E. Arnett, A. S. McClay, K. Shea, and A. K. McEarchern. 2005. Evaluation of ecological risk to populations of a threatened plant from an invasive biocontrol insect. Ecol. Appl. 15: 234–249.

Lynch, L. D., and M. B. Thomas. 2000. Nontarget effects in the biocontrol of insects with insects, nematodes, and microbial agents: the evidence. Biocontrol News Inform. 21 (4): 117N–130N.

Pemberton, R.W. 2000. Predictable risk to native plants in weed biological control. Oecologia 125: 489–494.

Simberloff, D., and P. Stiling. 1996. Risks of species introduced for biological control. Biol. Conserv. 78: 185–192.

Stiling, P. 2002. Potential non-target effects of a biological control agent, prickly pear moth, *Cactoblastis cactorum* (Berg.) (Lepidoptera: Pyralidae), in North America, and possible management actions. Biol. Invasions 4: 273–281.

Van Driesche, R. G., R. I. Carruthers, T. Center, M. S. Hoddle, J. Hough-Goldstein, L. Morin, L. Smith, D. L. Wagner et al. 2010. Classical biological control for the protection of natural ecosystems. Biol. Control Suppl. 1, S2–S33.

van Lenteren, J. C., J. S. Bale, F. Bigler, H. M. T. Hokkanen, and A. J. M. Loomans. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. Ann. Rev. Entomol. 51: 609–634.

# Session 5: Prospects for Enhancing Augmentative Releases of Beneficial Organisms Using Radiation

## Integrating augmentative biological control and inherited sterility for management of lepidopteran pests

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Insect pest management can benefit from the integration of biological control agents and the release of sterile insect pests (hosts). Released sterile or semi-sterile insects and their sterile progeny may augment natural enemies by serving as hosts for build-up of the natural enemies prior to the time the target pest has reached the economic threshold, and as hosts for natural enemy survival during critical times of low pest populations. Conversely, periodic releases of a natural enemy for the control of a pest population can enhance the efficacy of sterile insects being deployed in a Sterile Insect Technique (SIT) program by reducing the number of pests reaching the adult stage, and thereby increasing the sterile: fertile overflooding ratio. These synergistic effects realized when integrating augmentative biological control and sterile insects have been examined with population models (Barclay 1987; Carpenter 1993; Knipling 1992). The results from these models suggest that although releasing only natural enemies or only sterile insects would be effective, combined releases of both natural enemies and sterile insects would require fewer released insects overall but provide superior control of the pest population.

Because lepidopteran females generally are more sensitive to radiation than are males of the same species, the dose of radiation used in a SIT program is usually set to fully sterilize the females but allow males to remain only partially sterile. When partially sterile males mate with fertile females the radiation-induced deleterious effects are inherited by the $F_1$ generation. As a result, egg hatch is reduced and the resulting offspring are both sterile and predominately male. Therefore, as a result of this inherited sterility, $F_1$ sterile progeny (eggs – adults) produced in the field can be used to augment the production of natural enemies.

Successful integration of inherited sterility and parasitoid augmentation into a management approach should consider whether or not parasitoid strategies negatively impact irradiated insects and their progeny more than that of the wild population, and whether or not inherited sterility negatively impacts the efficacy and reproduction of parasitoids. Knowledge of any negative impact of inherited sterility on parasitoids would be important before implementing an area wide management program that combined these two tactics. For example, if parasitoids that attack the $F_1$ sterile progeny are unable to develop normally, and most of the hosts present are $F_1$ sterile progeny, then there could be a negative impact on subsequent parasitoid populations. Conversely, if parasitoids develop normally on $F_1$ eggs, larvae, and pupae, then the greater number of hosts available would allow for an increase in the parasitoid population. Because many hosts of the $F_1$ generation would experience genetically-induced mortality before they reached the adult stage, any parasitoids completing their development in these hosts would result in a positive and synergistic increase in the efficacy of an area wide management program (Carpenter 2000). Laboratory and field trials with several different lepidopteran species have indicated that $F_1$ sterile progeny were acceptable and suitable as hosts for egg and larval parasitoids, and female parasitoids demonstrated no ovipositional preference for progeny from female moths paired with either irradiated or un-irradiated males (Carpenter et al. 2005; Hendrichs et al. 2009). Other studies have revealed that releases of egg parasitoids along with releases of irradiated moths have provided synergistic suppression of moth pest populations (Carpenter et al. 2004; Saour 2009) as predicted by population models.

Although the synergistic effects realized through the simultaneous release of irradiated lepidopterans and their parasitoids are quite intriging, there are other scenerios in which inherited sterility could be integrated with natural enemies to suppress pest populations. The release of partially sterile males and females could produce large numbers of sterile $F_1$ eggs and larvae that could be field-reared on early season host plants. Natural enemies (native and/or released) could use the $F_1$ eggs, larvae and pupae as hosts and thereby substantially increase the natural enemy population for the next generation of the pest insect (Proshold et al. 1998). Any surviving sterile $F_1$ progeny would produce sterile adults that would negatively impact the next generation of the pest insect. Similarly, another management option could be the establishment of host plants for the pest in insecticide-free nursery crops adjacent to the crop to be protected. Nursery plants could be artificially infested with pest larvae to provide natural enemies (native and/or released) with an adequate supply of hosts. If the pest larvae used in the artificial infestations (nursery crops) are sterile (i.e., the progeny of irradiated parents), then non-parasitized larvae would not contribute to the increase of the wild pest population, but would produce sterile adults that would negatively impact the next generation of the pest insect (Carpenter 2000).

## References

Barclay, H. J. 1987. Models of sterile insect releases for populations under attack by parasitoids. Ecol. Mod. 36: 155-169.

Carpenter, J. E. 1993. Integration of inherited sterility and other pest management strategies for *Helicoverpa zea*, pp. 363-370. *In* Management of insect pests: nuclear and related molecular and genetic techniques. IAEA-STI PUB/909, Vienna, Austria.

Carpenter, J. E. 2000. Area-wide integration of lepidopteran $F_1$ sterility and augmentative biological control, pp. 193-200. *In* Ken-Hong Tan (ed.), Area-wide control of fruit flies and other insect pests. Penerbit University Sains Malaysia, Pulau Pinang, Malaysia.

Carpenter, J. E., S. Bloem, and J. H. Hofmeyr. 2004. Acceptability and suitability of eggs of false codling moth (Lepidoptera: Tortricidae) from irradiated parents to parasitism by *Trichogrammatoidea cryptophlebiae* (Hymenoptera: Trichogrammatidae). Biol. Control 30: 351-359.

Carpenter, J. E., S. Bloem, and F. Marec. 2005. Inherited sterility in insects, pp. 115-146. *In* V. A. Dyck, J. Hendrichs, and A. S. Robinson (eds.), Sterile Insect Technique, Principles and Practice in Area-Wide Integrated Pest Management. Springer, The Netherlands.

Hendrichs, J., K. Bloem, G. Hoch, J. E. Carpenter, P. Greany, and A. S. Robinson. 2009. Improving the cost-effectiveness, trade and safety of biological control for agricultural insect pests using nuclear techniques. Biocon. Sci. Technol. 19, S1: 3-22.

Knipling, E. F. 1992. Principles of insect parasitism analyzed from new perspectives: practical implications for regulating insect populations by biological means, Agric. Handbook # 693. USDA-ARS. Washington DC.

Proshold, F. I., H. R. Gross, and J. E. Carpenter. 1998. Inundative release of *Archytas marmoratus* (Diptera: Tachinidae) against the corn earworm and fall armyworm (Lepidoptera: Noctuidae) in whorl-stage corn. J. Entomol. Sci. 33: 241-255.

Saour, G. 2009. Effect of Early Oviposition Experience on Host Acceptance in *Trichogramma* (Hymenoptera: Trichogrammatidae) and Application of $F_1$ Sterility and *T. principium* to Suppress the Potato Tuber Moth (Lepidoptera: Gelechiidae). Biocon. Sci. Technol. 19, S1: 225-234.

# Current management efforts against *Cactoblastis cactorum* as a pest of North American prickly pear cactus, *Opuntia* spp.

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The unintentional arrival of *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) to Florida changed the scope of this celebrated weed biological control agent from savior to pest. *Cactoblastis cactorum* (Argentine cactus moth) is native to South America (Argentina, Uruguay, Paraguay, and southern Brazil) and their larvae feed gregariously inside stems of cactus in the genus *Opuntia* spp. (Cactaceae: Opuntioideae) (prickly pear cactus) (Mann 1969). Based on this insects' substantial control of non-native *Opuntia* spp. in Australia and other parts of the world (Dodd 1940; Pettey 1948; Zimmermann et al. 2000), concern has been raised for cactus-based agriculture and ecosystems in the southwestern USA and Mexico, and *Opuntia* biodiversity throughout the region (Zimmermann et al. 2004; Stiling 2002).

To stop the spread of *C. cactorum* along the Gulf of Mexico in USA, control tactics including sanitation and the Sterile Insect Technique (SIT) were developed, validated, and implemented (Bloem et al. 2007). In the SIT validation study, sanitation alone reduced moth populations, but sanitation coupled with the SIT lowered the population to immeasurable levels. Effective overflooding ratios (~10:1; sterile:wild male trap captures) and collections of eggsticks from wild female with sterile male matings indicated that sterile males were competitive with wild males. Implementation of the SIT area-wide technology lead to the complete eradication of this pest from barrier islands off the Mexican Yucatan Peninsula (NAPPO 2009), representing the first time a lepidopteran pest was eradicated from a country in the Western Hemisphere. Deployment of the SIT in the USA also eradicated *C. cactorum* from several barrier islands along the Gulf coast (Hight and Carpenter 2009). Unfortunately, the operational program to stop the westward spread of this insect with the SIT, sanitation, and host plant removal was curtailed in 2011.

With continued expansion of *C. cactorum*, especially into the desert Southwest, biological control may be the only self-perpetuating control option and practical approach to protecting the vast native *Opuntia* habitats. Surveys and life table studies in Florida identified a few natural enemies attacking the invasive *C. cactorum*, but no natural enemy has been identified to have an important impact on *C. cactorum,* or been host specific enough to warrant further study (Paraiso et al. 2011, unpublished data). In the native range of *C. cactorum*, several natural enemies had been identified, but their use as biological control agents was deemed limited (Pemberton and Cordo 2001). Previous information indicated that *Apanteles alexanderi* Brèthes (Hymenoptera: Braconidae) was an important natural enemy of *C. cactorum* in Argentina, but lacked host specificity since it was commonly found attacking at least seven other cactophagous lepidopterans (Pemberton and Cordo 2001). However, during more recent and extensive field surveys and life table studies in Argentina, two morphologically distinct Argentine *Apanteles* species were found emerging from cactophagous pyralid larvae (Martínez et al. 2012). Molecular and taxonomic studies confirmed species separation and a new species was described, *Apanteles opuntiarum* Martínez & Berta (Martínez et al. 2012). Field collections of cactophagous larvae were made in 16 Argentine Provinces and returned to the laboratory to evaluate parasitism. To date, over 33% of collected *C. cactorum* larvae were parasitized with *A. opuntiarum*.

*Cactoblastis doddi* Heinrich, whose only know host plant is *Opuntia sulphurea* Gillies ex Salm-Dyck, was also parasitized by *A. opuntiarum*. *Cactoblastis bucyrus* Dodd, whose host plant is the treelike cactus *Trichocereus atacamensis* (Philippi) W.T. Marshall & T.M. Bock (Cactaceae: Cactoideae), was parasitized by *A. alexanderi*. *Tucumania* sp. Dyar (Lepidoptera: Pyralidae), a solitary prickly pear feeding moth, was also parasitized by *A. alexanderi*. Host plants of *C. doddi* and *C. bucyrus* occur in the same habitat and insects were collected in close proximity to one another. Similarly, *C. cactorum* and *Tucumania* sp. are also found in the same habitat, often attacking the same individual host plant. Field collections identified *C. cactorum* and *C. doddi* to be hosts of *A. opuntiarum,* and *C. bucyrus* and *Tucumania* sp. to be hosts of *A. alexanderi*. Host specificity of *A. opuntiarum* will continue in Argentina on co-evolved cactophagous Lepidoptera and in a Florida quarantine facility on North American Lepidoptera, especially cactophagous pyralids.

A putative female sex pheromone for *C. cactorum* was identified by Heath et al. (2006) and is the basis for the currently used Argentine cactus moth monitoring trap (Bloem et al. 2005). More recently, the blend of chemicals comprising this attractant was re-evaluated and a 2-component lure may be more effective and less costly than the current 3-component lure. However, capture of non-target lepidopteran species by the *C. cactorum* lure cannot be significantly reduced because several Pyralidae and Noctuidae species use at least one of these pheromone components to lure mates earlier in the scotophase. In hopes of offering protection from *C. cactorum* attack to prickly pear plants under plantation settings, tests were initiated to evaluate the synthetic pheromone's potential for use in mating disruption. *Opuntia* plantations in Argentina were identified as field sites to conduct pheromone mating disruption trials. These plantations are infested with *C. cactorum*, are of sufficient size to establish a replicated study, and are logistically within range of our local technical support. Trapping surveys are being conducted to determine *C. cactorum* population size and to verify phenology. Trapping trials were initiated to examine the attractive space around a trap baited with a standard commercial lure. Preliminary results suggested that the active space around a trap was likely less than a 20 m radius. Additional trials to define trapping space will provide critical information regarding the spacing of mating disruption dispensers in the mating disruption trials. Laboratory trials were initiated to see if pheromone lures in a small closed cage (45×45×45 cm) would inhibit mating of *C. cactorum*. Preliminary data indicated that the incidence of mating was reduced as the number of pheromone lures was increased in the cage.

## References

Bloem, S., S.D. Hight, J.E. Carpenter, and K.A. Bloem. 2005. Development of the most effective trap to monitor the geographical expansion of the cactus moth *Cactoblastis cactorum* (Lepidoptera: Pyralidae). Florida Entomol. 88: 300-306.

Bloem, K., S. Bloem, J. Carpenter, S. Hight, J. Floyd, and H. Zimmermann. 2007. Don't let cacto blast us – development of a bi-national plan to stop the spread of the cactus moth *Cactoblastis cactorum* in North America, pp. 337-344. *In* M. J. B. Vreysen, A. S. Robinson, and J. Hendrichs (eds.), Area-Wide Control of Insects Pests: From Research to Field Implementation. Springer, Dordrecht, The Netherlands.

Dodd, A.P. 1940. The Biological Campaign Against Prickly Pear. Commonwealth Prickly Pear Board, Brisbane, Australia.

Heath, R.R., P.E.A. Teal, N.D. Epsky, B.D. Dueben, S.D. Hight, S. Bloem, J.E. Carpenter, T.J. Weissling, P.E. Kendra, J. Cibrian-Tovar, and K.A. Bloem. 2006. Pheromone-based attractant for males of *Cactoblastis cactorum* (Lepidoptera: Pyralidae). Environ. Entomol. 35: 1469-1476.

Hight, S.D., and J.E. Carpenter. 2009. Flight phenology of male *Cactoblastis cactorum* (Lepidoptera: Pyralidae) at different latitudes in the southeastern United States. Florida Entomol. 92: 208-216.

Mann, J. 1969. Cactus-feeding insects and mites. U.S. Nat. Mus. Bull. 256: v-x, 1-158.

Martínez, J.J., C. Berta, L. Varone, G. Logarzo, P. Zamudio, A. Zaldívar-Riverón, and R.G. Aguilar-Velasco. 2012. DNA barcoding and morphological identification of Argentine species of *Apanteles* (Hymenoptera: Braconidae), parasitoids of cactus-feeding moths (Lepidoptera: Pyralidae: Phycitinae), with description of a new species. Invertebrate Systematics 26: 435-444.

NAPPO (North American Plant Protection Organization). 2009. Detection and eradication of a cactus moth (*Cactoblastis cactorum* Berg) outbreak in Isla Contoy, municipality of Isla Mujeres, Quintana Roo, Mexico.
http://www.pestalert.org/oprDetail.cfm?oprID=376&keyword=cactoblastis%20cactorum
Last accessed 01/15/2013.

Paraiso, O., S.D. Hight, M.T.K. Kairo, and S. Bloem. 2011. Egg parasitoids attacking *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in North America. Florida Entomol. 94: 81-90.

Pemberton, R.W., and H.A. Cordo. 2001. Potential and risks of biological control of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in North America. Florida Entomol. 84: 513-526.

Pettey, F.W. 1948. The biological control of prickly pear in South Africa. Sci. Bull., Dept. of Agric. of the Union of South Africa 271: 1-163.

Stiling, P. 2002. Potential non-target effects of a biological control agent, prickly pear moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), in North America, and possible management actions. Biol. Invasions 4: 273-281.

Zimmermann, H.G., V.C. Moran, and J.H. Hoffmann. 2000. The renowned cactus moth, *Cactoblastis cactorum*: Its natural history and threat to native *Opuntia* in Mexico and the United States of America. Diversity Distrib. 6: 259-269.

Zimmermann, H., S. Bloem, and H. Klein. 2004. Biology, History, Threat, Surveillance and Control of the Cactus Moth, *Cactoblastis cactorum*. International Atomic Energy Agency, Vienna, Austria.

# Mobile mating disruption – the challenge of cross-species behavioural suppression

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Eradication of insects in urban environments is challenging for many reasons, including opposition to aerial application of biopesticides by the public (Suckling and Brockerhoff 2010; Gamble et al. 2010). The potential of a new application for mass reared sterile insects has been examined in laboratory and field trials in New Zealand, Australia and Hawaii, in order to develop a socially–acceptable solution for ground release of pheromone with self-dispersing point sources, or "mobile mating disruption". An earlier project attempted to interrupt fruit fly mating by placing highly attractive para-pheromones on a second mass-reared fruit fly species, and behavioral disruption was observed during the day, but flies mated at the normal rate at dusk (Suckling et al. 2007).

The next approach we have examined has used Mediterranean fruit flies *Ceratitis capitata* (Diptera: Tephritidae, medfly) against the invasive light brown apple moth, *Epiphyas postvittana* (Lepidoptera: Tortricidae). The stages identified as necessary for the project included "do no harm" to the vector, demonstration of attraction of the target moth pest by the pheromone-treated insect vectors, and finally field release of pheromone-treated medflies and suppression of the target moth pest measured as a reduction in trapping to synthetic and virgin female lures. A dose response of moth pheromone applied directly to insect cuticle revealed similar results from moths and flies (codling moth (*Cydia pomonella,* Lepidoptera: Tortricidae), light brown apple moth, housefly (*Musca domestica,* Diptera: Muscidae), blowfly (*Lucilia cuprina,* Diptera: Callipohoridae), medfly). Toxicity was evident only at very high doses. The dose of 100 µg of moth pheromone per insect was chosen as the upper limit to limit toxicity to the vector. Treatment of insect cuticle resulted in attraction of significant numbers of male moths into traps, but only for a short period in each case. This was not surprising given the volatility of the pheromone, and led to the examination of alternative approaches to achieve longevity, by formulation. Application of the pheromone to the insects in a micro-encapsulated formulation kindly provided by Suterra LLC (Bend, Oregon) was successful at prolonging the release of pheromone according to studies of moth attraction using treated insects. This approach was used in subsequent trials in Australia and New Zealand. This formulation also offered the advantage of reducing exposure to the moth pheromone through controlled release, and mortality of such pheromone-treated medflies was shown to be negligible.

Field trials in urban Perth, Australia were then conducted, using replicated four hectare plots with street access through the plot center and perimeter to enable trapping, using virgin-female moths and synthetic lures for assessment of communication disruption, a surrogate measure for population suppression. Medflies (Vienna 7/99, Temperature Sensitive Lethal genetic sexing strain, male only), were sprayed with micro-encapsulated two-component moth pheromone using a modified Potter tower, and kept in cardboard buckets with lids until daytime field release (Suckling et al. 2011). Approximately 3500 flies were release per ha (based on omni-directional dispersal from perimeter release on street corners). The results from pre-release sampling and comparison with control plots indicated healthy moth population throughout the trials (four releases in total), and significant disruption of attraction of male moths to virgin females and synthetic lures for several days. Fly longevity was limited to 2-3 days, although disruption lasted longer than this. There was evidence of flies grooming off the microencapsulated pheromone, and this is thought to partly explain the longer efficacy of disruption compared with fly longevity. Practical difficulties in scaling up to test the concept in plots larger than 4 ha have limited further development, as an engineering solution is needed to deliver the pheromone onto flies before release. Moths were caught in fruit fly traps, adding evidence to the interpretation of the mechanism (Suckling et al. 2011).

A second approach, of feeding unprotected pheromone in honey to flies was also examined (Jang et al. unpublished data). Flies fed with unformulated two component moth pheromone in honey were found to be attractive in field trials in Hawaii. The levels of disruption seen in field trials was significant and promising but not as effective as seen with micro-encapsulated pheromone. This is probably due to the lack of any mechanism to control the release rate. The recent identification of a more attractive four component sex pheromone blend for light brown apple moth offer potential for improving the results, especially if combined with micro-encapsulation to control the release rate. Recent trials with the four-component blend showed superior efficacy at communication disruption (Suckling et al. in press). It is possible that feeding flies with micro-encapsulated four-component pheromone blend before release would solve the logistical problem of fly treatment and improve results. The development of a new generic and socially-acceptable solution for exotic moth pests in urban environments could offer significant benefits, but more work is needed.

## Acknowledgements

This work was commenced during an OECD Fellowship (DMS) and supported by the United States Department of Agriculture, the CRC for Plants Biosecurity and Better Border Biosecurity (www.b3nz.org).

## References

Gamble, J. C., T. Payne, and B. Small. 2010. Interviews with New Zealand community stakeholders regarding acceptability of current or potential pest eradication technologies. N. Z. J. Crop Hort. Sci. 38: 57–68.

Suckling, D. M., and E. G. Brockerhoff. 2010. Invasion biology, ecology, and management of the light brown apple moth (Tortricidae). Ann. Rev. Entomol. 55: 285–306.

Suckling, D. M., E. B. Jang, L. A. Carvalho, J. T. Nagata, E. L. Schneider, and A. M. El-Sayed. 2007. Can ménage-à-trois be used for controlling insects? J. Chem. Ecol. 33: 1494–1504

Suckling, D. M., B. Woods, V. J. Mitchell, A. Twidle, I. Lacey, E. B. Jang, and A. R. Wallace. 2011. Mobile mating disruption of light-brown apple moths using pheromone-treated sterile Mediterranean fruit flies. Pest Man. Sci. 67: 1004–1014.

# Improving mass rearing and commercial shipments of *Spalangia endius* W. (Hymenoptera: Pteromalidae) through irradiation of its host

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The house fly, *Musca domestica* L. (Diptera: Muscidae) is a major problem in animal confined facilities (Geden and Hogsette 1994) causing nuisance, including to the neighborhood and carrying diseases that affect humans and animals (Talley et al. 2009; Greenberg 1971). High fly densities produce stress to poultry workers and hens affecting the economic value of their products (Learmount et al. 2002). The repetitive and inappropriate use of 2,2-dichlorovinyl dimethyl phosphate (DDVP), pyrethroids and cyromazine in poultry farms resulted in the appearance of resistance worldwide (Pinto and Prado 2001; Tang et al. 2002; Roca-Acevedo et al. 2009). This is the reason why actually biological control of flies with *Spalangia* spp. and *Muscidifurax* spp. within an integrated pest management perspective is seen as the most effective alternative for control (Zapater et al. 1994; Zapater et al. 2009).

In commercial insectaries a guarantee of fresh fly pupae is required as a host for the daily production of *Spalangia endius* Walker (Hymenoptera: Pteromalidae*),* but production variation or urgent demand of parasitoids result in unexpected demand for fresh pupae that cannot be normally supplied. The optimal parasitation age of pupae is between 24-48 h old, making it impossible to stockpile the host pupae. Sometimes, when different species/strains of parasitoids are reared, host pupae contamination results in a major problem. Another rearing consideration is that host pupae parasitation is not 100% when they are exposed to parasitoids, resulting in fly emergence some days later and requiring additional steps and insectary work in eliminating them as well as the empty puparia from the parasitized pupae.

The objective of this work was to try to prolong the suitability of house fly pupae for parasitism, and prevent the emergence of adult flies from non-parasitized pupae using a combination of gamma radiation, anoxia, and cold. Morgan et al. (1986) showed that no house fly adults emerged from pupae irradiated (gamma radiation) at a dose of 500 Gy, and that pupae irradiated at this dose were still good hosts for *S. endius*. They were also able to extend the suitability of pupae for parasitism to 8 weeks by storing them at 4.4ºC and adequate humidity. We extend this research and further describe the effect on *S. endius* fitness of irradiating house fly pupae and placing them in cold storage in anoxia for up to 2 months before using them as host material, under mass rearing conditions.

The colony of *S. endius* employed for these studies was established with wild insects collected from different areas from Argentina and the regular *M. domestica* lab colony. Studies were carried out in an environmental chamber maintained at 25 ± 1°C, L:D 14:10 h, and 70-85% RH. Irradiation of the house fly pupae was conducted at "IONICS", a commercial irradiation facility, using a Cobalt$^{60}$ irradiator with an activity level of (1942.5 x $10^{13}$ Bq (525,000 Ci). Because of the high dose rate, special procedures adjusting the exposure distance were developed by the staff at "IONICS" to ensure that an effective dose of only 500 Gy was delivered at a dose rate of 20 Gy/min.

The storage potential of irradiated pupae was calculated preparing 54 plastic bags of two hundred ml of 48-h-old (± 12 h) *M. domestica* pupae. The bags were hermetically sealed, causing the development of anoxia due to respiration of the pupae, and then irradiated with 500 Gy of gamma radiation. The bags were maintained permanently at 6 ± 0.5°C in a refrigerator. Every day for the first 8 days (days 0-7) and every third day for days 9 to 63, 1 sample of 50 pupae was extracted from each of 2 bags and the bags discarded. The pupae were introduced into the parasitization cage for two days with a ratio of 1 female parasitoid for every 4 pupae. The parasitized pupae were then passed to a vial to allow parasitoid emergence. A total of 9 replicates were conducted. Two bags of non-irradiated pupae on day 0 were exposed to parasitoids as control.

The fitness of the parasitoids reared on irradiated pupae held in cold storage in anoxia for 1, 20, 40 days and the control were evaluated through fecundity and longevity tests. The fecundity test evaluated the capacity to produce offspring of individual mated females that were daily exposed to fresh pupae during 1 day when they were 3 to 6 days old. Longevity was calculated by selecting individually females from each treatment provided with regular colony pupae until the females died. Three replicates were done for each treatment and experiments were carried out in appropriate vials and controlled environmental conditions.

Results indicated that house fly pupae could be maintained as parasitoid host in storage for increasing lengths of time. There was a decrease in the parasitism rate as the length of pupal storage time increased following the formula Y = 53.93 - 0.50X (Figure 1). The $R^2$ value for this equation was 0.51. In accordance with the equation, the rate of parasitism percentage decreased 25% after 26.5 d, 50% after 53.2 d and 58% near the end of the experiment 60 d later. This experiment was carried out under simulated mass rearing conditions, which probably accounts for the resulting variation in parasitism rates.

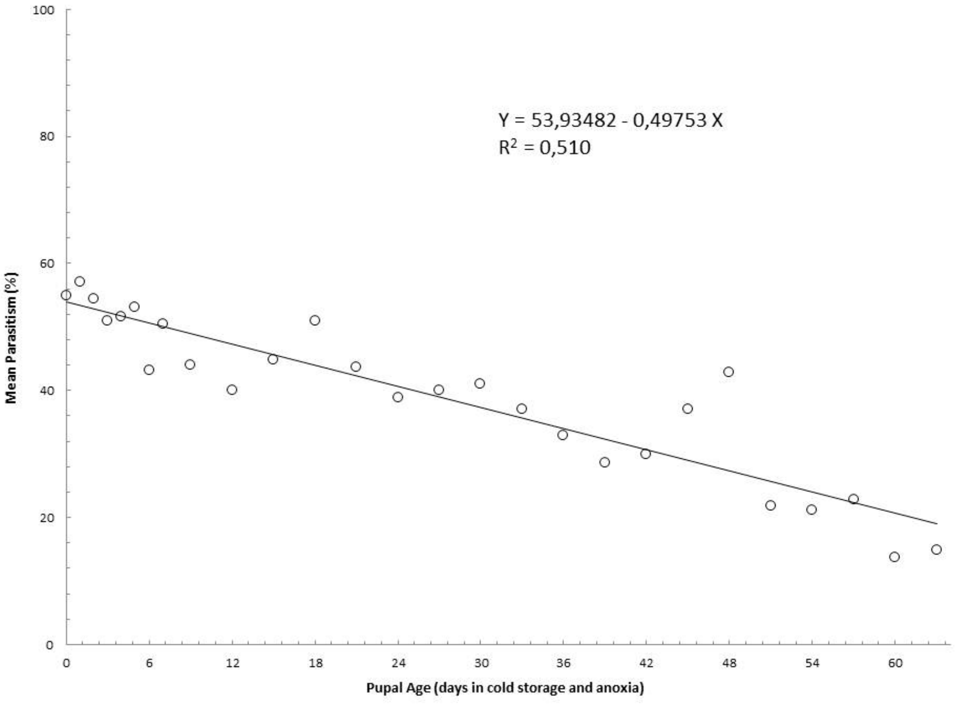

Figure 1. Mean percent parasitism by *S. endius* on irradiated *M. domestica* pupae that had been held in cold storage (6 ± 0.5°C) and anoxia for up to 63 d. N=9 replications.

The fecundity experiment compared the number of progeny produced per female reared from normal (control) pupae versus per female reared from irradiated pupae held in cold storage for 1, 20 or 40 days. A Kruskal-Wallis ANOVA test for ranks was employed and no significant differences in fecundity were found among females from the different treatments ($df$ = 3, $N$ = 179, $H$ = 1.279, $P$ = 0.169). Females produced an average of 12-14 offspring on day 3, which decreased to 5-6 on day 6.

The results of the longevity studies of adult female parasitoids emerging from normal (control) pupae and pupae that were stored in anoxia and cold for 1, 20 or 40 d was monitored for 16 days and cumulative daily averages plotted. A Kruskal-Wallis ANOVA test for ranks was applied and no significant differences among the 4 treatments were discovered ($df = 3$, $N = 179$, $H = 0.79$, $P < 0.852$). Fifty percent of the females had died by day 8 and 100% by day 16.

Results indicated that 500 Gy combined with anoxia and refrigeration can extend the suitability of house fly pupae for parasitism by *S. endius* to 30 d or more. For example, when irradiated pupae were used, the rate of parasitism based on progeny production decreased by 50% from about 60% to 30% after 53.2 d of pupal storage. The parasitoids that were produced in this manner from pupae stored up to 40 d were of good quality and lived as long and produced as many offspring as parasitoids reared from normal pupae. Thus, from a commercial standpoint, it should be possible to guarantee customers a specified number of quality adult parasitoids by appropriately adjusting the number of parasitized pupae that are sent depending on how long the pupae had been stored.

The use of irradiated host material for mass rearing parasitoids has a number of advantages. First, in the case of *S. endius* rearing, developing house fly pupae produce a significant amount of biological heat. As a result, pupae must be well spread out on trays when they are exposed to the parasitoids. However, if the pupae are irradiated, development is stopped and more pupae can be placed per unit area. Second, because not all of the exposed pupae are parasitized, current protocols require that the pupae must be held for 4 days to eliminate any flies that emerge before they can be shipped to customers. This costs both time and space. If irradiated pupae were used, parasitized pupae can be shipped sooner, which frees up holding space and creates more flexibility in the system for orders to be prepared, shipments to arrive, and parasitoids to be delivered to the field. The use of irradiated host material has already become standard practice in the mass rearing of fruit fly parasitoids in support of sterile insect release programs (Sivinski and Smittle 1990; Cancino et al. 2002). With proper management, a rotating stockpile of host pupae can be maintained such that when parasitoid demand is low, excess host material produced during that time can be put into storage. If parasitoid demand increased unexpectedly or an opportunity arises to develop new clients, pupae can be brought out of storage to quickly meet the need. The ability to store pupae can also reduce work, for example, during weekends and holidays. The use of gamma irradiation would also make it possible for insectaries to trade/sell irradiated host pupae amongst themselves instead of parasitized pupae, which occasionally occurs when demand exceeds production. This would allow them to continue to provide their customers with the same strain they normally provide.

## References

Cancino, J, R. L, Gómez, and J. Toledo. 2002. Irradiación de larvas de *Anastrepha ludens* (Loew) (Diptera: Tephritidae) para inhibir la emergencia de moscas en la cría del parasitoide *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Folia Entomol. Mex. 41: 195-208.

Geden, C. J., and J. A. Hogsette. 1994. Research and extension needs for integrated pest management of arthropods of veterinary importance. *In* Proceedings. of a workshop. 12-14 April 1994. Lincoln, NE. USDA-ARS, Gainesville, FL.

Greenberg, B. 1971. Flies and diseases, Princeton Univ. Press, Princeton, NJ.

Learmount, J., P. Chapman and A. Macnicoll. 2002. Impact of an insecticide strategy for house fly (Diptera: Muscidae) control in intensive animal units in the United Kigdom. J. Econ. Entomol. 95:1245-1250.

Morgan, P. B., Smittle B. J., and R. S. Patterson. 1986. Use of irradiated pupae to mass culture the microhymenopterous pupal parasitoid *Spalangia endius* Walker

(Hymenoptera: Pteromalidae). I. *Musca domestica* L. (Diptera: Muscidae). J. Entomol. Sci. 21: 222-227.

Pinto, M., and A. Prado. 2001. Resistance of *Musca domestica* L. populations to cyromazine in Brazil. Mem. Inst. Oswaldo Cruz 96:729-732.

Roca-Acevedo, G., A. C. Toloza, and M. C. Zapater. 2009. Insecticide resistance of house fly, *Musca domestica* (L.) from Argentina. Parasitol. Res. 105:489-493.

Sivinski, J., and B. Smittle. 1990. Effect of gamma radiation on the development of the Caribbean fruit fly *Anastrepha suspensa*, and the subsequent development of its parasite *Diachasmimorpha longicaudata* (Ashmead). Entomol. Exp. Applic. 55: 295-297.

Talley, J. l., A. C. Wayadande, L. P. Wasala, A. C. Gerry, J. Fletcher, U. DeSilva, and S. E. Gilliland. 2009. Association of *Escherichia coli* O157:H7 with filth flies (Muscidae and Calliphoridae) captured in leaf greens fields and experimental transmission of *E. coli* O157:H7 to spinach leaves by house flies (Diptera:Muscidae). J. Food Prot. 72:1547-1552.

Tang, J., M. Caprio, C. Sheppard, and D. Gaydon. 2002. Genetics and fitness costs of cyromazine resistance in the house fly (Diptera:Muscidae). J. Econ. Entomol. 95:1251-1260.

Zapater, M. C., C. Martinez-Rey, and J. Mazzoli. 1994. Control de moscas en establecimientos agropecuarios de cría intensiva: primer logro comercial del control biológico inundativo en la Argentina. Gaceta agronómica XIV N° 82:360-375.

Zapater, M.C., G. Perez Camargo, M. Gañet, I. Asurmendi, and M.S. Paladino. 2009. Control biológico y prácticas de manejo de la *Musca domestica* (Diptera: Muscidae) en galpones de gallinas ponedoras. Proceedings (CD). XII Jornadas Fitosanitarias Argentinas. Rio Hondo, Oct. 2, 2009.

# Integrating the SIT with parasitoids and pathogens against fruit fly pests: the potential of using sterile insects as vectors of pathogenic microorganisms

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Fruit flies are a pest complex of great economic importance that adversely affect the marketing of fruits and vegetables and the development of the horticultural industry worldwide (Aluja 1993). Their control requires an area-wide approach that includes the use of environmentally oriented strategies such as the application of selective toxic baits to suppress pest populations at specific sites, the augmentative releases of parasitoids in areas identified as reservoirs of fruit flies populations, and the application of the Sterile Insect Technique (SIT) to reduce the population growth rate (Montoya et al. 2007). The use of this technologicy has allowed the generation and maintenance of free and low prevalence zones in Mexico, which have significantly favored the international marketing of various fruit and vegetable products (Gutierrez 2010).

Current integrated pest management (IPM) programs are focused on a more sustainable approach in order to mitigate the adverse effects commonly associated with the indiscriminate use of pesticides (Montoya 2007). This reinforces the incorporation of Augmentative Biological Control (ABC) as a viable strategy in the integrated management of fruit flies, using the mass release of parasitoids in marginal areas that harbor high densities of alternate hosts and where the implementation of chemical control is not a socially, ecologically and economically appropriate alternative. The greatest strength of this approach lies in the integration of this type of biological control with the SIT, where synergistic results can be expected as two different biological states of the pest are attacked simultaneously (Knipling 1992). The final goal is to suppress sustainably the fruit fly populations in those areas identified as reservoirs and to facilitate the management of these pests in commercial orchards (Montoya et al. 2000, 2007), which provides the double benefit of efficient production of healthy fruits and also environmental protection.

A novel tool, the use of sterile flies as vectors of fungus spores, has been recently proposed by Toledo et al. (2007), Quezada-Moraga et al. (2008) and Dimbi et al. (2009). Tests in laboratory and field cages using Mexican fruit fly *Anastrepha ludens* (Loew) sterile males as vectors of *Beauveria bassiana* (Balsamo) Vuillemin spores showed that the mating success of inoculated males was not affected compared with the control group during three days post-inoculation. The horizontal transmission of the fungus spores to females during the first two days ranged between 80-84 % through matings, and 15-22 % through mating attempts and contact during courtship. The fertility of infected females was notably reduced and longevity did not extend beyond 15 days (Toledo et al. 2007). Novelo-Rincon et al. (2009) carried out field cage tests to determine the sexual competitiveness, copulation duration, and sperm transfer of sterile and wild *A. ludens* males that were treated or not with *B. bassiana* conidia immediately before mating tests. No significant differences in sexual competitiveness were found between untreated and conidia treated sterile males, indicating that the presence of conidia did not significantly reduce mating performance.

This approach was evaluated, recently under open field conditions against wild *Ceratitis capitata* (Wied.) populations (Flores et al. submitted). The sterile inoculated males were released by air using the chilled adult technique over 7,000 ha of coffee plantations in Guatemala at a rate of 3000 males/ha. The impacts of releases were determined using dry traps baited with a food attractant. The effects of these releases on *Apis mellifera* (honey bee) and *Hypothenemus hampei* (coffee berry borer) populations were also evaluated, as well on associated parasites and parasitoids. The results revealed that inoculated sterile males were able to transmit fungal spores to 44% of the wild *C. capitata* captured in the traps, which became infected through interactions that occurred during the formation of leks, mating or mating attempts. There was no transmission of the fungal spores to non-target insect species such as the coffee berry borer, honey bees or their parasites, demonstrating that this strategy is highly specific.

Results strongly suggest the possibility of using sterile flies as fungus vectors and/or in combination with parasitoid releases to sustainably control wild fruit fly populations. They also show that this strategy may constitute a safe, environmentally friendly and selective alternative for suppressing fruit fly pests under an area-wide IPM scheme.

## References

Aluja, M. 1993. *Manejo Integrado de Moscas de la Fruta.* Edit. Trillas. México D. F. 251p.

Dimbi, S., N. K. Maniania, and S. Ekesi. 2009. Effect of *Metarhizium anisopliae* on the inoculation behavior of three species of African tephritid fruit flies, *Ceratitis capitata*, *Ceratitis cosyra* and *Ceratitis fasciventris*. Bio. Control. 50: 111-116.

Flores, S., S. Campos, A. Villaseñor, W. Enkerlin, J. Toledo, P. Liedo, and P. Montoya. Sterile males of *Ceratitis capitata* (Dipt.: Tephritidae) as disseminators of *Beauveria bassiana* spores for IPM strategies. J. Econ. Entomol. (submitted).

Gutiérrez, J. M. 2010. El programa de moscas de la fruta en México, pp. 3-10. *In* Montoya, P., J. Toledo, and E. Hernández. (eds.) Moscas de la Fruta. Fundamentos y Procedimientos para su Manejo. S y G Editores, México, D.F.

Knipling, E. F. 1992. Principles of Insect Parasitism Analyzed from New Perspectives. Agriculture Handbook No. 693. ARS-USDA. Washington D. C. USA.

Montoya, P. 2007. Control biológico por aumento en el manejo integrado de moscas de la fruta, pp. 145-156. *In* V. Hernández-Ortiz (ed.). Moscas de la Fruta en Latinoamérica (Diptera: Tephritidae). Diversidad, Biología y Manejo. S y G editores, México D. F.

Montoya, P., P. Liedo, B. Benrey, J. F. Barrera, J. Cancino, J. Sivinski, and M. Aluja. 2000. Biological control of *Anastrepha* spp. (Diptera: Tephritidae) in mango orchards through augmentative releases of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Bio. Control. 18: 216-224.

Montoya, P., J. Cancino, M. Zenil, G. Santiago, and J. M. Gutiérrez. 2007. The augmentative biological control component in the Mexican campaign against *Anastrepha* spp. Fruit Flies, pp. 661-670. *In* Vreysen, M. J. B., A. S. Robinson, and J. Hendrichs (eds.) Area-Wide Control of Insect Pests: From Research to Field Implementation. Springer, Dordrecht, The Netherlands.

Novelo-Rincón, L. F., P. Montoya, V. Hernández-Ortiz, P. Liedo, and J. Toledo. 2009. Mating performance of sterile Mexican fruit fly *Anastrepha ludens* (Diptera: Tephritidae) males treated with *Beauveria bassiana* (Bals.) Vuill. J. App. Entomol. 133: 702-710.

Quesada-Moraga, E., I. Carballo-Martin, I. Jurado-Garrido, and C. Álvarez-Santiago. 2008. Horizontal transmission of *Metarhizium anisopliae* among laboratory populations of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Bio. Control. 47: 115-124.

Toledo, J., S. E. Campos, S. Flores, P. Liedo, J. F. Barrera, A. Villaseñor, and P. Montoya. 2007. Horizontal transmission of *Beauveria bassiana* in *Anastrepha ludens*

(Diptera: Tephritidae) under laboratory and field cages conditions. J. Econ. Entomol. 100: 291-297.

# Session 6: Mass Production of Natural Enemies: Challenges and Opportunities

## The ectoparasitoid *Tamarixia radiata* as a tool in IPM of the Asian citrus psyllid *Diaphorina citri*

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We are developing a mass rearing system for *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) using orange jessamine, *Murraya paniculata* (L.) Jack (*Rutaceae*) as a host for rearing the psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) (Gómez-Torres, 2009). The parasitoid was collected in Brazil in 2006 (Gómez-Torres et al., 2006). Unfortunately, in our country, growers are spraying intensively with neonicotinoids and pyrethroids. They are not using selective insecticides and as a consequence natural parasitization is lower now than in 2006. The most important restriction for the utilization of biological control is chemical spraying to control the vector (Parra et al., 2010). We are only releasing in non-citrus host, *M. paniculata*, areas, organic areas and in abandoned groves. We are producing approximately 50,000 parasitoids per month and releasing 400 per hectare. It is necessary to scale up the parasitoid rearing system in order to release it in huanglongbing problem areas (240,000 hectares in Brazil). We are obtaining good results in field releases. We have seen a reduction of between 50 to 93% of psyllid nymphs in some areas in the State of São Paulo (Figure 1).

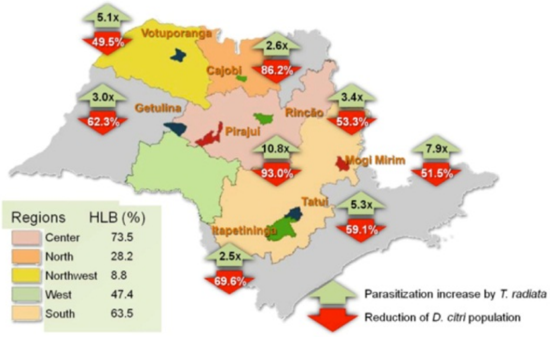

Figure 1. *Tamarixia radiata* release areas in São Paulo, Brazil.

### References

Gomez Tores, M.L., D.E. Nava, S. Gravena, V.A. Costa, and J.R.P. Parra. 2006. Registro de *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) em *Diaphorina citri* Kuwayama Hemiptera: Psyllidae) em São Paulo. Revista de Agricultura, 81: 112–117.

Gomez Torres, M. L. 2009. Estudos bioecológicos de *Tamarixia radiata* (Waterston, 1922) (Hymenoptera: Eulophidae) para o controle de *Diaphorina citri* Kuwayama, 1907 (Hemiptera: Psyllidae). Tese (Doutorado em Entomologia) – Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo.

Parra, J.R.P., J.R.S. Lopes, M.L.G. Torres, D.E. Nava, and P.E.B. Paiva. 2010. Bioecologia do vetor *Diaphorina citri* e transmissão de bactérias associadas ao huanglonbing. Citrus Research and Technology 31: 1–100.

# Adult mass rearing affects foraging behavior in the larval parasitoid *Microplitis croceipes*

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Parasitoid adults are normally reared in groups to ensure mating and to save time and space of rearing. However, parasitoid females are known to change their host foraging behavior in the presence of conspecifics. Foraging females change superparasitism rate, patch time and sex ratio in response to physical contact with conspecifics or chemicals left by conspecifics (King 1986, van Alphen and Visser, 1990, van Alphen et al. 1992, Darrouzet et al. 2008). Thus, adult mass-rearing of parasitoids may have a strong influence on their subsequent behavior and physiology.

*Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) is an endo-larval parasitoid of *Helicoverpa/Heliothis* spp. ( Lepidoptera: Noctuidae) in North America (Lewis and Burton 1970, Le and Takasu 2005). Takasu and Le (2007) found that mass-reared *M. croceipes* females often attack and oviposit in conspecific adults. The parasitoid eggs laid in abdominal cavity of conspecific adults hatched with less than 30 % of time, but died without molting to second instar. The adults attacked by conspecifics significantly reduced their longevity. However, it is unknown whether individually reared females do such attack to conspecific adults.

Although *M. croceipes* females are capable of discriminating parasitized hosts from unparasitized hosts (Wäckers 1994), it is often observed that they oviposit in parasitized hosts as often as unparasitized (Tillman and Powel, 1992). In the previous studies, however, mass-reared females were used for the host discrimination experiments. Therefore, frequent superparasitism by this parasitoid may be caused by mass-rearing condition.

In the present study, we compared conspecific attack and superparasitism between individually reared females reared and mass-reared females to determine if adult mass rearing changes subsequent foraging behavior of *M. croceipes*. We also examined the possibility that increasing number of eggs in the calyx increases ovipositional motivation and thus conspecific attack and superparasitism.

## Materials and Methods

*Microplitis croceipes* were maintained with third instar of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) as hosts after Le and Takasu (2005). Soon after emergence, 20 pairs of males and females were kept for two days or four days in a rearing cage (30 X 30 X 30 cm) and used for experiments. These females were designated as mass-reared females. Individually reared females were individually reared in a plastic cup (10 cm in diameter, 6cm in height) since 5-6 hours after emergence until experiments.

To compare conspecific attack rate between mass-reared females and individually reared females, mass-reared or individually reared females were allowed to contact the small amount of frass that was placed at the center of the filter paper (9cm in diameter), and then observed if conspecific attack occurred.

To compare superparasitism rate between mass-reared females and individually reared females, mass-reared or individually reared females that had oviposited three hosts were given an unparasitized third instar host, a host parasitized by themselves, or a host parasitized by a conspecific on the filter paper and then observed if oviposition occurred.

## Results and Discussion

When females contacted the host frass, they intensively antennated it and sometimes probed it with their ovipositor. When two mass-reared females that were stimulated by host frass met on the filter paper, they always stung each other, whether they were naïve or had ovipositional experience. However, when two individually reared females encountered, only less than 40 % of them stung another female. There was a significant difference in conspecific attack rate between mass-reared females and individually reared females, whether they were 2-day-old or 4-day-old, or whether they were naïve or experienced ($\chi^2$ test, $P<0.05$).

There was no significant difference in the percentage of self superparasitism between mass-reared females and individually reared females ($\chi^2$ test, $P>0.05$). However, the percentage of conspecific superparasitism by mass-reared females was significantly greater than that by individually reared females ($\chi^2$ test, $P<0.05$). Mass-reared females had more mature eggs in the calyx than individually reared females (t-test, $P<0.05$).

We showed in the present study that adult mass rearing increases conspecific attack and superparasitism in *M. croceipes*. It seems that mass rearing conditions such as physical contacts with conspecifics stimulate accumulation of mature eggs in the calyx. Since the calyx is the space where mature eggs are temporally stored before being deposited, it may be reasonable that accumulation of mature eggs in the calyx increases the motivation to oviposit in this species. The increased motivation to oviposit may cause mass-reared females to attack conspecifics or to superparasitize more frequently. Adult mass rearing effects must be taken into account when conducting behavioral or physiological studies or field release of parasitoids in biological control programs.

## References

Darrouzet, E., G. Boivin, and C. Chevrier. 2008. Sex allocation decision under superparasitism by the parasitoid wasp *Eupelmus vuilleti* J. Insect Behav.

King, B. H. 1987. Offspring sex ratios in parasitic wasps. Quart. Rev. Biol. 62:367-396.

Le, K. H., and K. Takasu. 2005. *Helicoverpa armiger*a as an alternative host of the larval parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). Appl. Entomol. Zool. 40:679-686.

Lewis, W. J., and R. I. Burton. 1970. Rearing *Microplitis croceipes* in the laboratory with *Heliothis zea* as hosts. J. Econ. Entomol. 61:673-676.

Takasu, K., and K. H. Le. 2007. The larval parasitoid *Microplitis croceipes* oviposits in conspecific adults. Naturwissenschaften 94:200-206.

Tillman, P. G., and J. E. Powell, 1992. Intraspecific host discrimination and larval competition in *Microplitis croceipes*, *Microplitis demolitor*, *Cotesia kazak* (Hym.: Braconidae) and *Hyposoter didymator* (Hym. Ichneumonidae), parasitoids of *Heliothis virescens* (Lep.: Noctuidae). Entomophaga 37:229-237.

van Alphen, J. J. M., and M. E. Visser. 1990. Superparasitism as an adaptive strategy for insect parasitoids. Ann. Rev. Entomol. 35:59-79.

van Alphen, J. J. M., M. E. Visser, and H. W. Nell. 1992. Adaptive superparasitism and patch time allocation in insect parasitoids: Searching in groups versus sequential patch visits. Func. Ecol. 6:528-535.

Wäckers, F. L. 1994. Multisensory foraging by hymenopterous parasitoids. Thesis, Wageningen Agricultural Univ. Wageningen, The Netherlands.

# Development and reproduction of the zoophytophagous predators *Orius thripoborus* and *Orius naivashae* on factitious prey and plant foods

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Predatory bugs of the genus *Orius* (Hemiptera: Anthocoridae) are important natural enemies of thrips and other pests throughout the world (Riudavets 1995, Hernández and Stonedahl 1999) and have been widely used in biological control programs (van den Meiracker and Ramakers 1991, Van Lenteren et al. 1997). *Orius thripoborus* (Hesse) and *Orius naivashae* (Poppius) (Hemiptera: Anthocoridae) are commonly found in eastern African cropping systems harboring various thrips species (Hernández and Stonedahl 1999) but have received little study. In South Africa, both pirate bugs are candidate biological control agents of economically important thrips pests, such as the sugarcane thrips *Fulmekiola serrata* (Kobus), the citrus thrips *Scirtothrips aurantii* Faure, the two avocado related thrips species *Heliothrips haemorrhoidalis* (Bouché) and *Selenothrips rubrocinctus* (Giard), and the western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Hesse 1940, Dennil 1992, Way et al. 2006, EPPO 2012). In order to optimize and rationalize the mass production and augmentative release of these zoophytophagous predators, it is crucial to understand their nutritional ecology. Important aspects of a successful mass rearing system for these *Orius* spp. include the availability of alternative food sources, adequate water sources, and suitable oviposition and living substrates.

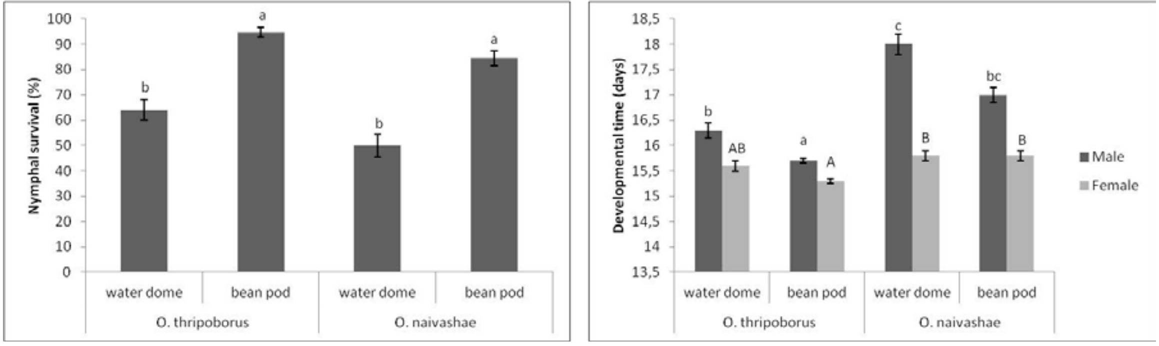

Figure 1. Nymphal survival (left) and developmental time (right) of *Orius thripoborus* and *Orius naivashae* on two moisture sources.

In a first experiment, the effect of two water sources on the developmental performance of *O. thripoborus* and *O. naivashae* was assessed in the laboratory. In the first treatment, water was provided in hemispherical domes (70 µL) made of Parafilm M using an encapsulation device. In the second treatment, a flat green bean pod, *Phaseolus vulgaris* L. (*Fabaceae)*, was used as a source of water. Frozen eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were supplied as food in both treatments. All experiments were done in climatic cabinets set at 23 ± 1 °C, 65 ± 5 % RH, and a photoperiod of 16h:8h (L:D). Survival rate of nymphs of both *O. thripoborus* and *O. naivashae* fed *E. kuehniella* eggs was about 30 % higher when a bean pod was offered as a water source than when a water dome was offered (Figure 1). A green bean pod as a moisture source also yielded faster male development for both *Orius* species, as compared with free water encapsulated in Parafilm (Figure 1). Adult weights, on the other hand, were not influenced by the offered moisture source and ranged from 0.31 to 0.33 mg for males and from 0.36 to 0.43 mg for females. These findings suggest that the anthocorids may derive supplemental nutrients from the bean pod.

Table 1. Developmental parameters and lifetime oviposition of *Orius thripoborus* and *Orius naivashae* on three diets.

| Species | Diet | Nymphal survival (%) | Female developmental time (days) | Female weight (mg) | Sex ratio[a] (♂:♀) | Lifetime oviposition | Female longevity (days) |
|---|---|---|---|---|---|---|---|
| *Orius thripoborus* | *E. kuehniella* | 86.6 ± 3.1a | 15.5 ± 0.1a | 0.36 ± 0.007ab | 1:1.15 | 122.5 ± 21.7a | 61,1 ± 5,6a |
| | *A. franciscana* | 87.0 ± 3.2a | 17.1 ± 0.2b | 0.37 ± 0.007ab | 1:0.81 | 110.3 ± 17.9a | 52,6 ± 3,8a |
| | Pollen | 66.3 ± 4.7b | 19.1 ± 0.3c | 0.33 ± 0.01a | 1:1.09 | 56.7 ± 11.6ac | 58,5 ± 6,5a |
| *Orius naivashae* | *E. kuehniella* | 85.5 ± 3.3a | 15.5 ± 0.1a | 0.45 ± 0.007c | 1:4.56* | 68.2 ± 9.9ab | 53,9 ± 2,7a |
| | *A. franciscana* | 79.1 ± 3.9a | 19.2 ± 0.2c | 0.38 ± 0.007b | 1:3.83* | 36.6 ± 8.3bc | 50,7 ± 2,6a |
| | Pollen | 77.9 ± 4.0ab | 20.6 ± 0.2d | 0.34 ± 0.007a | 1:2.11* | 17.6 ± 3.9c | 50,5 ± 4,4a |

Means ± SE within a column followed by the same letter are not significantly different ($P > 0.05$; Tukey (female longevity), Tamhane (female adult weight and lifetime oviposition), Mann-Whitney U (female developmental time) or probit test (nymphal survival))

[a] Values with an asterisk differ significantly from a 1:1 ratio ($P < 0.05$; Chi-square test)

In a second experiment, three foods were tested on both *Orius* species: two factitious prey types and one plant diet. The first factitious food consisted of frozen eggs of *E. kuehniella*. A second factitious food consisted of hydrated decapsulated cysts of the brine shrimp *Artemia franciscana* Kellogg (Anostraca: Artemiidae), originating from Great Salt Lake (Utah, USA). The plant diet was composed of moist frozen honey bee pollen. In each treatment, a flat green bean pod was provided as a water source, substrate (hiding place), and extra nutrient source. Both *Orius* species were able to complete their development on moist honey bee pollen. However, with nymphal survival percentages of 66 % for *O. thripoborus* and 78 % for *O. naivashae*, mortality on honey bee pollen was higher than on the tested factitious foods (Table 1). Development of females of either species was faster on the factitious foods than on pollen (Table 1). Predators fed flour moth eggs had shorter developmental times than those fed brine shrimp cysts (Table 1). Female body weights of both species were generally similar, except when the predators were offered *E. kuehniella* eggs and green beans, resulting in heavier body weights for *O. naivashae* (Table 1). Although for *O. thripoborus* sex ratios in all treatments were essentially 1:1, skewed sex ratios in *O. naivashae* were observed (Table 1). So far, it is not clear which mechanisms are involved in the female biased sex ratios observed in our *O. naivashae* population. Females of *O. thripoborus* and *O. naivashae* were able to produce viable eggs on all diets. Overall, females of both species fed pollen laid 26 - 51 % of the number of eggs deposited by those fed *E. kuehniella* eggs or *A. franciscana* cysts. Regardless of diet, *O. thripoborus* produced more eggs than *O. naivashae* (Table 1). Longevity of females varied between 50,5 and 61,1 days and did not differ among treatments (Table 1).

Our findings indicate that both *O. thripoborus* and *O. naivashae* are easily produced in the laboratory using factitious foods. Furthermore, a pollen diet alone allowed part of the predators to reach adulthood and reproduce. Developmental and reproductive performance of *O. thripoborus* was superior to that of *O. naivashae*, with a faster nymphal development, shorter preoviposition period, and overall better fecundity. *Orius thripoborus* also performed better on pollen than *O. naivashae*. These findings suggest that *O. thripoborus* has greater potential than *O. naivashae* for use in biological control programs, but other factors like diapause, temperature preferences, searching behavior, predation capacity, and habitat and prey preference may provide a more complete picture of the effectiveness of both predators.

## References

Dennil, G. B. 1992. *Orius thripoborus* (Anthocoridae), a potential biocontrol agent of *Heliothrips haemorrhoidalis* and *Selenothrips rubrocinctus* (Thripidae) on avocado fruit in the Eastern Transvaal. S. A. Avocado Growers' Assoc. Yrb. 15: 55–56.

(EPPO) European and Mediterranean Plant Protection Organization. 2012. PQR - EPPO database on quarantine pests (available online). http://www.eppo.int.

Hernández, L. M., and G. M. Stonedahl. 1999. A review of the economically important species of the genus *Orius* (Heteroptera : Anthocoridae) in East Africa. J. Nat. Hist. 33: 543–568.

Hesse, A. J. 1940. A new species of *Thripleps* (Hemiptera-Heteroptera, Anthocoridae) predaceous of the citrus thrips (*Scirtothrips aurantii* Faure) in the Transvaal. J. Entomol. Soc. S. Afr. 3: 66–71.

Riudavets, J. 1995. Predators of *Frankliniella occidentalis* (Perg.) and *Thrips tabaci* Lind.: a review, pp. 43-87. In A. J. M. Loomans, J. C. Van Lenteren, M. G. Tommasini, S. Maini and J. Riudavets (eds). Biological Control of Thrips Pests. Wageningen Agricultural University, Wageningen, the Netherlands.

van den Meiracker R. A. F., and P. M. J. Ramakers. 1991. Biological control of the western flower thrips *Frankliniella occidentalis*, in sweet pepper, with the anthocorid predator *Orius insidiosus*. Med. Fac. Landb., Rijksuniv. Gent 56: 241–249.

van Lenteren J. C., M. M. Roskam, and R. Timmer. 1997. Commercial mass production and pricing of organisms for biological control of pests in Europe. Biol. Control 10: 143-149.

Way, M. J., M. Stiller, G. W. Leslie, D. E. Conlong, M. G. Keeping, and R. S. Rutherford. 2006. *Fulmekiola serrata* (Kobus) (Thysanoptera : Thripidae), a new pest in southern African sugarcane. Afr. Entomol. 14: 401–403.

# Performance of *Amblyseius swirskii* Athias-Henriot and *Amblydromalus limonicus* Garman (Mesostigmata: Phytoseiidae) on factitious foods and pollen

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## Introduction

The generalist predatory mites *Amblyseius swirskii* Athias-Henriot and *Amblydromalus limonicus* Garman (Mesostigmata: Phytoseiidae) are economically important biological control agents of several key pests in greenhouses, such as thrips and whiteflies (Messelink et al. 2006). Pollen or factitious food sources can be used to give certain arthropod predators a head-start upon release or to support existing populations in the crop so as to reduce the frequency of releases. Factitious food sources can also be used in the mass production of the predators. Eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) have shown to be a suitable factitious food for several insect predators including lady beetles (De Clercq et al. 2005), anthocorid bugs (Vacante et al. 1997, Arijs and De Clercq 2001), mirid bugs (Fauvel et al. 1987, Castañé et al. 2006) and lacewings (Cohen and Smith 1998). Cysts of the brine shrimp *Artemia* sp. (Anostraca: Artemiidae) are a another type of factitious food with potential for the production of a number of insect predators, including *Orius* spp. (Hemiptera: Anthocoridae) (Arijs and De Clercq 2001) and *Macrolophus* spp. (Hemiptera: Miridae) (Castañé et al. 2006).

Conceivably, these factitious foods could also be useful to support the growth and reproduction of generalist predatory mites like *A. swirskii* and *A. limonicus*. In the present study, we compared the developmental and reproductive performance of *A. swirskii* and *A. limonicus* when fed on *E. kuehniella* eggs and decapsulated dry cysts of the brine shrimp *Artemia franciscana* Kellogg (Anostraca: Artemiidae) with that on cattail, *Typha latifolia* L. (*Typhaceae*) pollen.

## Materials and Methods

Stock colonies of *A. swirskii* and *A. limonicus* were initiated from specimens supplied by Biobest N.V. (Westerlo, Belgium) and Koppert B.V. (Berkel en Rodenrijs, The Netherlands), respectively. Mites were reared on plastic arenas and cattail pollen (*Typha latifolia* L.) was dusted on the arenas every other day as a food source. To examine the development and reproduction of individual *A. swirskii* and *A. limonicus* mites, modified Munger cells were used as rearing microcosms (Ogawa and Osakabe 2008). A single predatory mite egg (<8h old) was placed in each cell. From the larval stage on, the microcosms were supplied with either frozen *E. kuehniella* eggs, dry decapsulated *A. franciscana* cysts or frozen *T. latifolia* pollen; foods were replenished every other day. Observations were made every 24 hours until all individuals had reached adulthood. After completing immature development, each female was paired with a male that was reared on the same diet as the female. Adults were observed daily to monitor their survival and reproduction. The experiments were done in a growth chamber at 23 ± 1 °C, 65 ± 5% RH and a 16:8 h (L:D) photoperiod.

## Results and discussion

Both phytoseiids showed high immature survival rates (90-100% survival) on all three diets, except *A. limonicus* when fed on *E. kuehniella* eggs (66% mortality). The diets had a significant effect on the total developmental time of *A. swirskii* females. Females fed on *T. latifolia* had the shortest developmental time while those fed on *E. kuehniella* had the slowest development. In *A. limonicus,* the fastest development of females was obtained when fed on *A. franciscana* cysts. Apparently, the eggs of *E. kuehniella* were difficult to handle for *A. limonicus* larvae as most of the mortality occurred during the larval stage. Likewise, some *A. swirskii* protonymphs had difficulty piercing the eggs, resulting in a greater variation in developmental time. For both *A. swirskii* and *A. limonicus* the daily oviposition rate of females reared on *A. franciscana* and *E. kuehniella* was significantly higher than that of females reared on *T. latifolia* pollen (Figure 1). The oviposition rate of *A. swirskii* did not differ among *A. franciscana* and *E. kuehniella* and averaged 1.5 eggs/female/day. This value is superior to that of mites reared on the natural prey *Frankliniella occidentalis* (Pergande) and *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) (Wimmer et al. 2008) (0.92 and 0.99 eggs/female/day, respectively) but lower than that of females fed on tomato russet mite *Aculops lycopersici* (Massee) (Trombidiformes: Eriophyidae) (Park et al. 2011) (1.9 eggs/female/day). When fed on *A. franciscana* or *E. kuehniella,* the oviposition rate of *A. limonicus* was about 80% higher than that of females supplied with *T. latifolia* pollen (1.3 eggs/female/day). van Houten et al. (1995) reported a slightly higher oviposition rate when *A. limonicus* was provided with sweet pepper pollen (1.5 eggs/female/day) as compared to our result with *T. latifolia* pollen. Diet did not have a significant effect on the longevity of both *A. swirskii* and *A. limonicus*.

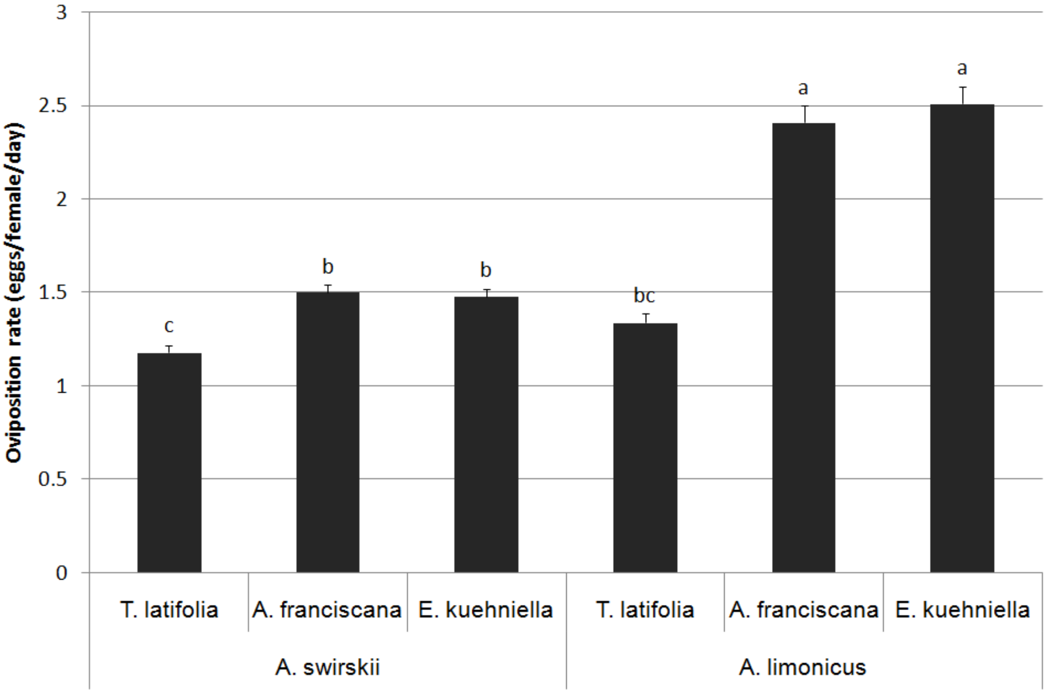

Figure 1. Mean (±SE) oviposition rate of *A. swirskii* and *A. limonicus* females fed on three diets at 23°C. Bars with the same letter are not significantly different (P>0.05).

Our results suggest that *A. franciscana* cysts and *E. kuehniella* eggs have potential as factitious food sources to support populations of *A. swirskii* and *A. limonicus* in the laboratory. Greenhouse studies are necessary to confirm whether these factitious foods can also effectively sustain populations of both phytoseiids in the crop in periods of prey scarcity.

## References

Arijs, Y., and P. De Clercq. 2001. Rearing *Orius laevigatus* on cysts of the brine shrimp *Artemia franciscana*. Biol. Control 21: 79–83.

Castañé, C., R. Quero, and J. Riudavets. 2006. The brine shrimp *Artemia* sp. as alternative prey for rearing the predatory bug *Macrolophus caliginosus*. Biol. Control 38: 405-412.

Cohen, A. C., and L. K. Smith. 1998. A new concept in artificial diets for *Chrysoperla rufilabris*: The efficacy of solid diets. Biol. Control 13: 49–54.

De Clercq, P., M. Bonte, K. Van Speybroeck, K. Bolckmans, and K. Deforce. 2005. Development and reproduction of *Adalia bipunctata* (Coleoptera: Coccinellidae) on eggs of *Ephestia kuehniella* (Lepidoptera: Phycitidae) and pollen. Pest Manag. Sci. 61: 1129–1132.

Fauvel, G., J. Malausa, and B. Kaspar. 1987. Etude en laboratoire des principales caracteristiques biologiques de *Macrolophus caliginosus* (Heteroptera: Miridae). BioControl 32: 529–543.

Messelink, G. J., S. E. F. Van Steenpaal, and P. M. J. Ramakers. 2006. Evaluation of phytoseiid predators for control of western flower thrips on greenhouse cucumber. BioControl 51: 753–768.

Ogawa, Y., and M. Osakabe. 2008. Development, long-term survival, and the maintenance of fertility in *Neoseiulus californicus* (Acari: Phytoseiidae) reared on an artificial diet. Exp. Appl. Acarol. 45: 123–136.

Park, H. H., L. Shipp, R. Buitenhuis, and J. J. Ahn. 2011. Life history parameters of a commercially available *Amblyseius swirskii* (Acari: Phytoseiidae) fed on cattail (*Typha latifolia*) pollen and tomato russet mite (*Aculops lycopersici*). J. Asia-Pacific Entomol. 14: 497–501.

Vacante, V., G. Cocuzza, P. De Clercq, M. Van De Veire, and L. Tirry. 1997. Development and survival of *Orius albidipennis* and *O. laevigatus* (Het.: Anthocoridae) on various diets. BioControl 42: 493–498.

van Houten, Y. M., P. C. J. van Rijn, L. K. Tanigoshi, P. van Stratum, and J. Bruin. 1995. Preselection of predatory mites to improve year-round biological control of western flower thrips in greenhouse crops. Entomol. Exp. Appl. 74: 225–234.

Wimmer, D., D. Hoffmann, and P. Schausberger. 2008. Prey suitability of western flower thrips, *Frankliniella occidentalis*, and onion thrips, *Thrips tabaci*, for the predatory mite *Amblyseius swirskii*. Biocontrol Sci. Technol.18: 541–550.

# Demogenetic processes at play in natural enemy mass productions: can we detect and manage them?

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Inoculative and inundative biological control strategies rely on perennial rearing of biological control agents (BCA) populations. From their collection in their native areas to their field releases, these populations undergo a number of events: variations of population size, environments, hosts, etc. Because of BCA short development times, these events likely induce evolutionary processes that can impact the mean and variance of BCA phenotypic traits and hence their performances in the rearing and/or in the field (Sorensen et al. 2012).

The short-term evolution of reared populations can notably be affected by various types of interacting "demogenetic" processes:

- Density-dependence, which refers to all those processes resulting in a positive (e.g. Allee effect) or negative (e.g. intraspecific competition) relation between population size and population growth rate.
- Demographic stochasticity, i.e. the departure from the expected population size estimated from individual or populational parameters (like survival, fecundity or sex ratio).
- Environmental stochasticity, defined as temporal fluctuations in the average population growth rates caused by variations in the abiotic or biotic conditions that cannot be captured by deterministic dynamics.
- Genetic processes, which regroup a numerous factors (genome structure, reproduction mode, population genetic diversity, genetic or symbiotic load, etc.) and processes (genetic drift, natural selection) that interactions can lead to a vast range of consequences such as adaptive allele loss, inbreeding or outbreeding depression, sex ratio biases, etc.

The impacts of these processes on the establishment success and evolution of small, introduced populations have recently been reviewed by Fauvergue et al. (2012). Actually, most patterns and issues highlighted in this review appear relevant to mass-reared BCA populations that were all introduced in industrial settings at least once in their recent history and that frequently undergo demographic bottlenecks more or less wanted and controlled by BCA producers.

Although the above-mentioned processes are rather well understood theoretically, evidence of their occurrence and impacts in reared BCA populations is still insufficiently documented. Evidence actually comes from a limited number of experimental works describing population evolution at neutral loci or in characters presumably under strong genetic control (reviewed in Hopper & Roush 1993, Roderick & Navajas 2003, Hufbauer & Roderick 2005) or "anecdotes" pinpointing certain facets of the evolution of mass-reared populations. In parallel, BCA producers have often developed empirical practices (e.g. rejuvenation of BCA strains) in order to circumvent possible undesired evolution. To our knowledge, the actual added-value of such practices has never been precisely evaluated. In fact, most of the documented cases of demogenetic processes affecting reared populations come from other research areas, e.g. from results documenting artificial selection on “model” or “domesticated” insect species (e.g. *Drosophila*, Sgro & Partridge 2000).

More precisely, the impacts of demogenetic factors are strongly expected in at least four situations. First, industrial practices can lead to drastic decreases in genetic diversity that might limit BCA adaptive performance on the target pests in the field. Second, these practices can cause inadvertent selection, e.g. for shorter development time and/or earlier fecundity that can impact other correlated life-history traits. This mechanism is currently studied in the harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), a species that has been widely used for biological control until some of its populations became invasive worldwide in the early 2000’s (Facon et al. 2011). Third, the maintenance of perennial rearings in uniform conditions is also supposed to have major impacts on the evolution of phenotypic trait reaction norms, and hence on the population phenotypic plasticity. The extent to which adaptation to homogeneous conditions (e.g. constant temperatures) may prevent efficiency in heterogeneous ecosystems including stressful conditions is still not known. Finally, another example of factor impacting reared populations is undoubtedly the presence, genetic diversity and infection polymorphism of endosymbionts. Cytoplasmic incompatibility inducers have been repeatedly documented in BCA populations (Engelstaedter and Hurst 2009, Vasquez et al. 2011). Their transmission mode and physiology can severely affect the phenotypes and demographic parameters (growth rate, sex-ratio) of their hosts. For instance, in a recent biological control program against the olive fruit fly in France using the endoparasitoid *Psyttalia lounsburyi* (Silvestri) (Hymenoptera : Braconidae), *Wolbachia* (Rickettsiaceae) endosymbionts have been suspected to, on the one hand, limit the possibility of genetic improvement through hybridization and, on the other hand, reduce individual fitness (Cheyppe-Buchmann et al. 2011), making the use of antibiotic treatments mandatory.

Until now, the scarcity of cases focusing on demogenetic processes in reared BCA populations impedes the precise evaluation of their evolutionary and economic impacts. Yet, standardized experimental designs and procedures could be adopted to document these two facets. Moreover, the broader availability of molecular-based tools, together with the outbreak of Next-Generation Sequencing technologies should also make easier and cheaper the use of genetic-marker-assisted experiments. Based on the characterisation of demogenetic processes in reared populations, numerous counter-measures could be developed and commonly implemented by BCA producers: e.g. antibiotic treatments in species displaying endosymbionts, purge of deleterious alleles in populations carrying particularly strong genetic load, selection of BCA populations displaying lowest extents of demographic stochasticity, genetic-marker-assisted choice of field-collected BCA populations to start new rearings or improve some existing ones through hybridization, etc.

In conclusion, we emphasize the need of more collaborative research between evolutionary biologists and BCA producers in order to 1) understand actual forces at play in mass-reared BCA populations; 2) adjust effective and economically sustainable procedures to circumvent undesirable impacts. In order to start such collaborations, our teams have started an international research project involving three academic institutes and three enterprises producing BCA: the project “COLBICS” (FP7 IAPP program).

## References

Cheyppe-Buchmann, S., M.-C. Bon, S. Warot, W. Jones, T. Malausa, X. Fauvergue, and N. Ris. 2011. Molecular characterization of *Psyttalia lounsburyi*, a candidate biocontrol agent of the olive fruit fly, and its *Wolbachia* symbionts as a pre-requisite for future intraspecific hybridization. BioControl 56: 713–724.

Engelstaedter, J., and G. D. D. Hurst. 2009. The ecology and evolution of microbes that manipulate host reproduction. Annu. Rev. of Ecol. Evol. S. 40: 127–149.

Facon, B., R. A. Hufbauer, A. Tayeh, A. Loiseau, E. Lombaert, R. Vitalis, T. Guillemaud, J. G. Lundgren, and A. Estoup. 2011. Inbreeding depression is purged in the invasive insect *Harmonia axyridis*. Curr. Biol. 21: 424–427.

Fauvergue, X., E. Vercken, T. Malausa, and R. Hufbauer. 2012. The biology of small, introduced populations, with special reference to biological control. Evol. Appl. 5: 424–443.

Hopper, K., R. Roush, and W. Powell. 1993. Management of genetics of biological control introductions. Annu. Rev. Entomol. 38: 27–51.

Hufbauer, R., and G. Roderick. 2005. Microevolution in biological control: mechanisms, patterns, and processes. Biol. Control 35: 227–239.

Roderick, G. K., and M. Navajas. 2003. Genes in new environments: genetics and evolution in biological control. Nat. Rev. Genet. 4: 889–899.

Sgro, C. M., and L. Partridge. 2000. Evolutionary responses of the life history of wild-caught Drosophila melanogaster to two standard methods of laboratory culture. Am. Nat. 156: 341–353.

Sorensen, J. G., M. F. Addison, and J. S. Terblanche. 2012. Mass-rearing of insects for pest management: challenges, synergies and advances from evolutionary physiology. Crop Prot. 38: 87–94.

Vasquez, C. J., R. Stouthamer, G. Jeong, and J. G. Morse. 2011. Discovery of a CI-inducing *Wolbachia* and its associated fitness costs in the biological control agent *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae). Biol. Control 58: 192–198.

# Session 7: Strategies to Increase the Adoption of Biological Control in Greenhouses

## Biological control in greenhouse pests in Chile

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It is a well-known fact that pests and diseases are frequently controlled in greenhouse production with a variety of pesticides. This has led to a growing concern about pesticide use, the presence of residues and worker risk associated to working in this environment. These concerns together with the successful use of biological control of pests in the greenhouse industry in Europe have stimulated initial development of biological control programs in Chile. In this discussion, we will focus our attention on the biological control of two pests.

### Greenhouse whitefly, *Trialeurodes vaporariorum*

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) is possibly the most important greenhouse pest in Chile over the past 15 years. Its importance started to increase from the late 1970s, possibly linked to the gradual development of resistance to insecticides (Vargas y Alvear, 2000). Chemical control is problematic, and growers are experiencing losses due to sooty mold, possibly reduced crop production, and increasing control costs. First observations of biological control were likely made Mr. S. Rojas, at INIA La Cruz about 40 years ago.

In relation to *T. vaporariorum* Rojas (2005), indicated that the parasitoids *Eretmocerus corni* Haldeman, *Encarsia lycopersici* De Santis and *Encarsia haitiensis* Dozier (Hymenoptera: Aphelinidae) are frequently observed in greenhouse tomato plants, and *Encarsia porteri* (Mercet) (Hymenoptera: Aphelinidae) is the most frequent parasitoid outside the greenhouse. *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) was collected in Chile for the first time in 1986 (Rojas, 2005), and also introduced from Canada in 1989. Together with Patricia Estay, Rojas evaluated this parasitoid in greenhouses. Results from their work were later transferred from INIA to the Insectary Biocontrol Ltda. This insectary started its commercial production in 2004, giving birth to the use of biological control in greenhouse production.

There were many obstacles during the initial production and use, since the available technology was rudimentary. Some of the initial problems observed with the use of *E. formosa* were:

- The lack of timely provision of insects from the rearing operation, generating:
    - Inadequate timing of the release
    - Too few parasitoids released
- Unsuitable environmental conditions for the parasitoid
- Use of non-selective insecticides
- High Initial densities of the pest and / or delayed release
- Lack of adequate local research

Successive improvements in the rearing method derived from the European expertise lead to an increase in the area under contract for biological control.

An insect that has been mentioned several times as a predator of the greenhouse whitefly and the tomato moth is Macrolophus sp. (Hemiptera: Miridae), present on tobacco plants. Samples of bryocorine mirids collected recently by the author in Quillota, Chile were sent to Dr. J. Antonio Sanchez (Spain). Dr. Sanchez preliminarily identify this insect as belonging to the Tupiocoris genus (Hemiptera: Miridae). Tobacco plants are often placed on the perimeter of greenhouses, aimed at increasing the availability of these insects. These tobacco plants exhibit high densities of Tupiocoris sp., especially inside the tobacco flowers, however there is no clear evidence that they feed on arthropod hosts and/or plant tissues. It is interesting to note that there are eight commercial producers of Macrolophus caliginosus (now Macrolophus pigmaeus) in Spain (Anonimous, 2012), suggesting this insect should be studied in Chile, especially because it is recommended against white flies, tomato moth, spider mites, thrips, etc.

## Tomato moth, *Tuta absoluta*

The tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a pest that damages, foliage, flowers, stems and fruits and this is capable of severely affecting the production. Natural enemies (parasitoids) associated with this pest in Chile are mentioned by Rojas (2005), and they belong to the following genera: *Trichogramma*, *Encarsia*, *Apanteles*, *Copidosoma*, *Horismenus*, *Cirrospilus* and *Dineulophus*. Currently the most important parasitoid in greenhouse and field grown tomato is *Apanteles gelechiidivoris* (Marsh) (Hymenoptera: Braconidae), which was sent from the north of the country, Azapa (Arica and Parinacota Region) in the 1982-83 season and from Colombia in 1984 and 1986, to INIA La Cruz.

The adult *A. gelechiidivoris* emerges from the cocoon that the moth larva constructs to pupate, which occurs usually on the soil. This life history characteristic can make it difficult to assess the impact of this natural enemy on the pest. Preliminary observations generated from Quillota greenhouses revealed a combination of the natural enemy with low use of insecticides resulted in 50 % of moth larvae being parasitized (observations of the author).

Tests performed by INIA La Cruz in the 80's, with the egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) obtained from Colombia, showed maximum values circa 35% parasitism of eggs which resulted in only a slight decrease in the damage caused by larvae to the fruit. Similar values were obtained later by P. Estay (personal communication) with *T. pretiosum*, *T. nerudai* Pintureau & Gerding and *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae). Regarding entomopathogens, isolates of *Metarhizium* spp. (Clavicipitaceae) and *Beauveria* spp. (Cordycipitaceae) from INIA Quilamapu, show potential for organic production (Estay and Bruna, 2002). Rodriguez et al (2006), evaluated entomopathogens in greenhouse tomato showing similar control as traditional insecticides. The use of commercial *Bacillus thuringiensis* Berliner (Bacillaceae) reduces larval abundance by about 50%. One of the effective isolates is BETK-03® which was isolated and is produced in Chile.

Other common pests in greenhouses crops in Chile are:

- Western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)
- Leaf miner *Liriomyza huidobrensis* (Blanchard), *Liriomyza quadrata* (Malloch) (Diptera: Agromyzidae)
- Mites, *Tetranychus urticae* (Koch), *Tetranychus cinnabarinus* (Boisduval) (Trombidiformes: Tetranychidae)
- Aphids *Aphis gossypii* Glover, *Aulacorthum solani* (Kaltenbach), *Myzus persicae* (Sulzer), *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae)

In some of these species, as is the case of leaf miners, natural enemies occur naturally. However, the use of pesticides often decreases the abundance of these natural enemies and hence their effectiveness to successfully control the target pest.

For a number of other cases, natural enemies are available for purchase from commercial insectaries to Chilean greenhouse growers. A summary of insectary-reared natural enemies that are most commonly used by growers is provided in the table below.

Table 1. * Production of natural enemies used in Chilean greenhouse production.

| Company | Natural enemies | Pest controlled | Current Situation |
|---|---|---|---|
| Biocontrol | *E. formosa*<br>*Eretmocerus* sp<br>*Macrolophus* sp | *T. vaporariorum* | 2004-2008<br>Currently not in production |
| Biobee | *Orius laevigatus*<br>*Phytoseiulus persimilis*<br>*Aphidius colemani*<br>*Diglyphus* | *Frankliniella occidentalis*<br>*Tetranichidae*<br>*Aphididae*<br>*Lyriomiza sp* | Products imported from Europe<br>http://www.biobee.cl/ |
| Control Best | *E. formosa*<br>*Eretmocerus* sp | *T. vaporariorum* | Initial production<br>http://www.controlbest.cl/ |
| Rojasi | *E. formosa*<br>*E. lycopersici*<br>*E. porteri* | *T. vaporariorum* | From 2006<br>www.rojasi.cl/ |
| Biobichos | *E. formosa* | *T. vaporariorum* | Initial production<br>http://www.biobichoschile.cl/ |

* Information provided by the companies

**Final comments**

Available strategies that can be integrated with biological control in pest management are:

- The use of soaps for control of adult whiteflies on tomatoes
- The trapping of tomato moth males with a high density of pheromone traps (P. Estay personal communication)
- The use of insect growth regulators (IGR) such as Buprofezin for white fly control
- Use of anti aphid mesh in greenhouse production

Overall, the use of biological control is very limited in the Chilean greenhouse production, among other factors, due to:

- Limited development of commercial breeding methods in local companies
- Almost no research on biological control of pests is actually carried out
- Lack of a variety of natural enemies against the pest complex affecting greenhouse production
- The absence of a subsidy to Biological Control use such as in Europe
- The Chilean greenhouse production is not aimed at export, reducing the pressure on the presence of residues in the final product
- The difficulty of regulating environmental conditions in plastic greenhouses (extreme temperatures)
- The perception of the producer of an unfavorable cost / benefit for use of biological control, considering the amount of training required and the effectiveness achieved
- Variability in the outcome of BC and more widespread coverage and attention that unsuccessful cases receive

- Insufficient information on the effect of pesticides on natural enemies (selectivity)

Moreover, the use of biological control in the near future can be promoted by:

- The use of pollinators, Bombus spp. (Hymenoptera: Apidae), in greenhouses restricts the use of pesticides
- Increasing research in this area
- Evaluate natural enemies not present in Chile, being used in Europe against tomato moth and whitefly
- The global trend to reduce pesticide residues in food
- Presence of foreign companies in Chile with extensive experience in production and use of natural enemies in greenhouse crops and especially the support of experienced professionals in the use of biological control method (Koppert and Biobee).

**Acknowledgments**. Special thanks for the useful comments are expressed to:
Andrés Alvear (XILEMA), Dennis Navea (CONTROLBEST), Patricia Estay (INIA), Marcos Gerding (BIOBICHOS), José Luis Paniagua (BIOCONTROL) and Adrian Wagner (BIOBEE)

## References

Anonimous, 2012. Organismos de Control Biológico. Consejería de Agricultura y Pesca. Dirección General de la Producción Agrícola y Ganadera. Accessed on July 2012. Available in http://dgpa.besana.es/ocb/listadoCultivoPlaga.do?cultivo=1&plaga=7

Estay, P., and A. Bruna. 2002. Insectos, acaros y enfermedades asociadas al tomate en Chile. Colección de libros INIA Nº 7..

Rodriguez, M., M. Gerding, and A. France. 2006. Efectividad de aislamientos de hongos entomopatógenos sobre larvas de polilla del tomate Tuta absoluta Meyrick (Lepidoptera: Gelechidae). Agricultura Técnica (Chile) 66: 159–165

Rojas, S. 2005. Control Biológico de plagas en Chile. Historia y avances. Colección de Libros INIA Nº 12. 123pp.

Vargas M., R. and A. Alvear de la F., 2000. Determinación de la susceptibilidad en tres poblaciones de Trialeurodes vaporariorum Westwood (Hemiptera: Aleyrodidae) a metomilo y buprofezin. Agric. Téc. [online]. 2000, vol.60, n.4, pp. 341–349.

# Banker plants, trap crops and other bioprotection developments in Canadian greenhouse floriculture

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## Introduction

Uptake of biological control by the greenhouse floriculture industry has historically lagged behind that of the greenhouse vegetable industry. Several factors have hindered implementation: tolerance for cosmetic damage and presence of pests is extremely low, a greater diversity of ornamental plants is grown requiring more complex control programs which may need to be tailored to a particular crop and environment, intensive use of pesticides, and quarantine restrictions on exported plants (Parrella, 1990). In Canada, though, a major shift has occurred over the past 10 years and biological control increasingly forms the foundation of IPM programs. Reasons for this include the advent of compatible pesticides, development of resistance in key pests, the loss/failure of registered insecticides, and access to new biological control products (Murphy et al. 2011).

Resistance and lack of effective products for western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), in particular forced many Canadian growers to place greater reliance on biological control (Murphy et al. 2011). Once deemed primary impediments to the use of natural enemies in floriculture, the ‘thrips factors’ became primary drivers to adoption. In fact, the ability to manage thrips biologically has become the key to the wider utilization of beneficials against other greenhouse pests. However, cost and efficacy are still essential considerations. A single biological control agent (BCA) (the pesticide paradigm) rarely provides satisfactory levels of control. Strategic selection and use of several natural enemies together, combined with innovative approaches to enhance their effectiveness, can deliver more efficacious and economic solutions.

Here, we will briefly examine the use of banker plants and trap crops to aid the establishment and performance of beneficial species, with specific reference to methods being evaluated in Canada to promote biological control for WFT. Further, we will provide updates on trials to investigate interactions between microbial and arthropod BCAs to enable their efficient concurrent use, and other key developments to aid the implementation of sustainable, biologically-based IPM strategies in Canadian floriculture.

## Trap plants

The principle behind the use of trap plants lies in the fact that most pests demonstrate a preference for particular plant species, cultivars or growth stages. Trap plants need to be more attractive than the main crop to prevent colonization of the crop, thereby reducing damage and (potentially) concentrating the insects into a limited area, providing a focal point for the application of control strategies (Shelton and Badenes-Perez, 2006).

WFT adults preferentially orient toward and land on flowering plants (Yudin et al., 1988), and will remain on the plant until the resource is exhausted (Rhainds and Shipp, 2003). Yellow flowering chrysanthemums, *Chrysanthemum morifolium* Ramat.

(*Asteraceae*) are more attractive to adult WFT than vegetative, bud and cracked-bud chrysanthemums (Buitenhuis and Shipp, 2006). Use of flowering chrysanthemums as trap plants lowered the number of adult WFT in a vegetative chrysanthemum crop and reduced crop damage (Buitenhuis et al., 2007). As these trap plants are more effective at intercepting dispersing WFT than attracting adults already present on the crop, they are likely to be most efficiently utilized when WFT are moving into or through the greenhouse (Buitenhuis and Shipp, 2006).

At low population density, trap plants are an excellent way to monitor thrips (indicator plants). As numbers increase, trap plants remove a significant proportion of thrips from the crop. Towards the end of the growing cycle, or if thrips populations are too high, additional control methods (biological or chemical) may be needed. By concentrating thrips on trap plants, they can be more readily managed using IPM tools. This practice is successfully used by Ontario growers of crops such as potted chrysanthemum (in their vegetative growth stage) and organic herbs. Blocks of flowering varieties known to be attractive to thrips are placed throughout the cropping area and are removed, destroyed and replaced every 2 weeks, before thrips leave the trap plants as flowers start to decline.

### Banker plants

Banker plants serve as ‘in-house’ rearing units for beneficial species. Established within the cropping area, they provide a continuous supply of BCAs for sustained pest management over a growing cycle. The plants supply necessary resources (e.g. prey and/or pollen, oviposition sites, stable habitat) that enable prolonged production of the natural enemy in the absence of these resources in the crop. Banker plant systems can deliver multiple benefits, e.g. reduced cost for biological control, enhanced quality and performance of a BCA, opportunities to implement a preventative pest management strategy, and their ready incorporation into IPM programs (Huang et al., 2011).

Widely used in greenhouse vegetable crops in Canada, the insidious flower bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), is an excellent WFT predator. Use in greenhouse ornamentals is limited, though because *Orius* can be slow to establish – a critical factor in short-term crops – and relatively poor control is achieved. In addition, the eggs of the predatory bug are removed from the greenhouse when the crop is shipped, so that predator populations do not build up in production areas (Waite et al., 2011). A good banker plant system for *Orius* must augment the diet of the predator in the absence of prey, and support reproduction and oviposition. Furthermore, the system must enable a high proportion of nymphs to survive through to adulthood and support population growth. Commercial ornamental growers in Ontario have had mixed success with the Black Pearl ornamental pepper, *Capsicum annuum* L. (*Solanaceae*), as a banker plant for *Orius*, prompting research to look for a better alternative. Comparing the Purple Flash ornamental pepper to Black Pearl, Waite et al. (2011) showed that *Orius* showed no preference in terms of egg-laying on these two varieties, and survival and development time of nymphs was also similar. However, Purple Flash supported greater population growth, possibly due to the higher number of flowers per plant providing *Orius* with access to superior nutritional resources (pollen).

### Integration

Growers frequently use multiple BCAs, without understanding how they may affect each other, or whether such combinations significantly enhance the level of control achieved. We need to understand these interactions to design more efficient IPM programs where different biologicals are used together in the most compatible and cost-effective manner.

In lab tests, the soil-dwelling thrips predator *Dalotia (Atheta) coriaria* (Kraatz) (Coleoptera: Staphylinidae) was shown to be susceptible to two commercial mycoinsecticides. In spite of this apparent incompatibility, thrips control was significantly improved in cage trials when *Dalotia* was used together with the same products (Brownbridge et al. unpubl.). Thus, we need to consider the biological significance of such interactions and devise crop-appropriate use practices for BCAs that include integrated strategies towards their successful deployment.

WFT has two distinct phases in its life cycle – foliar and soil-dwelling – which may be targeted with BCAs. *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) is increasingly used to control soil-dwelling pupae, while a *Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae) spray has the potential to infect WFT in the foliage. When both control agents were applied together, WFT feeding damage was significantly reduced, as the treatments impacted all life stages (Brownbridge et al., 2011). Similarly, thrips populations were suppressed below action threshold levels when *Metarhizium brunneum* Petch (Clavicipitaceae) (soil treatment) was used together with a weekly nematode drench; likewise, when the fungus was used together with predatory mites, e.g. *Strateolaelaps scimitus* (Wormersley) (Mesostigmata: Laelapidae), significantly better levels of control were obtained. These findings provide insight into the way forward in floriculture where use of more than one BCA is undoubtedly required to achieve the necessary levels of control and damage prevention.

Novel preventative application strategies for microbials also need to be examined. Imported from offshore production facilities into Canada, chrysanthemum cuttings are frequently infested with thrips, at times in high numbers. Romero et al. (2011) showed that by immersing the cuttings in suspensions of *B. bassiana* or *S. feltiae* prior to propagation, thrips were significantly reduced. Such techniques have the potential to limit ‘starting’ populations of these pests, reduce pest pressures thereafter, and increase opportunities to successfully use other preventative biological control tactics.

## Conclusion

We are on the threshold of a new era for biological control in protected horticulture. In Canada, commercial growers have demonstrated that this approach can be effectively used on a large scale. The ability to control thrips is opening the door to broader adoption of biological control. Innovative and creative approaches to the application and utilization of suites of natural enemies, together with supporting business cases that clearly demonstrate the economic advantages to using such systems, will further promote their uptake.

## References

Brownbridge, M., T. Saito, R. Buitenhuis, A. Brommit, and G. Murphy. 2011. Developing a biologically-based IPM program for western flower thrips, *Frankliniella occidentalis*, in greenhouse floriculture. IOBC/wprs Bulletin 68: 21-24.

Buitenhuis R., and J. L. Shipp. 2006. Factors influencing the use of trap plants for the control of *Frankliniella occidentalis* (Thysanoptera: Thripidae) on greenhouse potted chrysanthemum. Environ. Entomol. 35: 1411–1416.

Buitenhuis, R., J. L. Shipp, S. Jandricic, G. Murphy, and M. Short. 2007. Effectiveness of insecticide-treated and non-treated trap plants for the management of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in greenhouse ornamentals. Pest Manag. Sci. 63: 910-917.

Huang, N., A. Enkegaard, L. S. Osborne, P. M. J. Ramakers, G. J. Messelink, J. Pijnakker, and G. Murphy. 2011. The banker plant method in biological control. Crit. Rev. Plant Sci. 30: 259-278.

Murphy, G. D., C. Gates, and G. R. Watson. 2011. An update on the use of biological control in greenhouse ornamental crops in Canada. IOBC/wprs Bulletin 68: 125-128.

Parrella, M. P. 1990. Biological control in ornamentals: Status and perspectives. IOBC/wprs Bulletin, 13(5): 161-168.

Rhainds M. and J. L., Shipp 2003. Dispersal of adult western flower thrips (Thysanoptera: Thripidae) on chrysanthemum plants: impact of feeding-induced senescence of inflorescences. Environ. Entomol. 32:1056–1065.

Romero, W., C. D. Scott-Dupree, G. Murphy, T. Blom, and C. R. Harris. 2011. Immersion treatments for imported chrysanthemum cuttings to control western flower thrips, *Frankliniella occidentalis* (Pergande) in greenhouses. IOBC/wprs Bulletin 68: 163-167.

Shelton, A. M., and F. R. Badenes-Perez. 2006. Concepts and applications of trap cropping in pest management. Ann. Rev. Entomol. 51: 285-308.

Waite, M. O., C. D. Scott-Dupree, M. Brownbridge, R. Buitenhuis, and G. Murphy. 2011. Evaluation of potential *Orius insidiosus* banker plants for western flower thrips biocontrol in ornamental crops. IOBC/wprs Bulletin 68: 189-192.

Yudin, L. S., B. E. Tabashnik, J. J. Cho, and W. C. Mitchell. 1988. Colonization of weeds and lettuce by thrips (Thysanoptera: Thripidae). Environ. Entomol. 17: 522-526.

# Genetic variation in insectary-produced biological control species

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## Introduction

Genetic diversity is thought to be essential in allowing a population to adapt to its surroundings and remain a competitive and effective species (Baker et al. 2003, Hopper et al. 1993). In insectaries, genetic variation may potentially be reduced or lost as a result of bottlenecks, assortative mating, genetic drift, and/or inbreeding (Hopper et al. 1993). Reduced genetic variation in insectary-produced natural enemies could make them ineffective for field applications. Without a certain degree of genetic variation, natural enemies may not adapt well to environmental changes (or to diverse release sites) or to subtle variation in their hosts' traits. Though a population with little to no variation may have achieved maximal fitness in a static, stable environment, they may not be useful once the diverse demands of field applications are placed on them. If they cannot perform their life history duties well, insectaries, clients, and crops suffer. With the significant costs caused by pest organisms, as well as the cost of pesticides (both monetarily and environmentally) augmentative biological control is an optimal mode of pest management, and optimal insects for this task are thus desired. If there is a strong need for adaptation between a biological control species and its host or a biological control species and its environment in order for it to perform optimally, preserving genetic diversity is important (Roderick et al. 2003).

Our study aimed to shed some light on the state of genetic variation of biological control agents within insectaries. Using a diploid predator, green lacewing *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae), and a haplodiploid parasitoid, wasp *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae),we assessed differences in genetic diversity between and among distinct insectary and wild-caught sources. As a result of one or more of the micro-evolutionary forces outlined above, we expect insectary-produced individuals to possess less genomic variation than wild-caught specimens because of the loss of allelic differences within rearing facilities over time.

## Materials & Methods

*Study System.* The study organisms, a haplodiploid parasitoid wasp (*D. isaea*) and a diploid predator green lacewing (*C. rufilabris*), were selected as representatives of two distinct life histories. Both species attack important pests and both are reared commercially. Wild-caught specimens of *C. rufilabris* were collected from Warren County, Iowa, USA and Burleson County, Texas, USA. The Iowa samples were collected at one site over the span of two weeks and Texas samples were collected at one site in a single night. Sampling took place in June of 2012. Insectary-produced samples of *C. rufilabris* were donated by two insectaries in North America and shipped live in June of 2012. Five adult female and five adult male lacewings from each of the four sources were extracted and used for AFLP analysis. *Chrysoperla plorabunda* (Fitch) (Neuroptera: Chrysopidae), the next most common wild-caught species of lacewing in the US, was used as an outgroup for genetic analyses to detect any incorrectly keyed wild-caught lacewings.

Wild-caught specimens of *D. isaea* were not found in great enough numbers to make statistically significant statements, so a comparison of genetic variation across four insectaries was done. Eighteen females from each insectary were used in the study. These were donated from distinct insectary sources (two where in North America). Six wild-caught *D. isaea* wasps (two diploid female, four haploid male) were found in sample material sent from D.N. Negev, from Israel and analyzed to have a rough idea of potential variation in wild populations.

*DNA Extraction, AFLP Development, and AFLP Analysis.* Whole genomic DNA was extracted using the DNeasy blood and tissue kit (QIAGEN Sciences, Maryland, USA) following the manufacturer's instructions. Amplified fragment length polymorphism (AFLP) markers were developed according to the protocol described by Vos et al. (1995). Primers *Eco*RI-ACT and *Mse*I-CAA were used for selective amplification. PCRs were performed using a GeneAmp® 9700 PCR system (Applied Biosystems, Forest City, CA). Samples were analyzed through capillary electrophoresis using an ABI 3130 genetic analyzer (Applied Biosystems, Forest City, CA, USA). AFLP results were analyzed in GeneMapper® 4.0 (Applied Biosystems, Forest City, CA). All individuals were used in the analysis (none were obvious outliers). As expected, the outgroup used for the *Chrysoperla* analysis proved to be significantly different, and all taxonomic identifications were verified by internationally recognized experts (NAMES, INSTITUTION). AFLP Surv® was used to calculate genetic diversity statistics, total $F_{st}$ values, and heterozygosity between our different populations. STRUCTURE v.2.3.3 (Falush et al. 2007) was used to identify genetic clusters.

## Results

AFLP analysis of 44 *C. rufilabris* and 79 *D. isaea* with the primer combination *Eco*RI-ACT and *Mse*I-CAA produced 194 bands for *C. rufilabris* and 87 bands for *D. isaea*. After markers were developed and allelic data were analyzed, AFLP Surv® generated several indices of genetic diversity. Total $F_{st}$ for *C. rufilabris* and *D. isaea* indicated "*little*" ($F_{st}$ =XXX, P=XXX) and "*moderate*" ($F_{st}$ =XXX, P=XXX) levels of genetic diversity, respectively (Hartl and Clark 1997). The p-value associated with the $F_{st}$ for *D. isaea* was not significant, making the result of "moderate" levels of diversity non-conclusive. Bayesian clustering analysis performed in STRUCTURE v.2.3.3 (Pritchard et al. 2010) revealed that all individuals were assigned with relatively equal probability to both hypothetical populations when K was set to 2 (Figure 1) indicating lack of population structure. Thus, the most probable number of populations was K = 1. Figure 1 shows homogeneity across all locations, insectary and wild-caught alike.

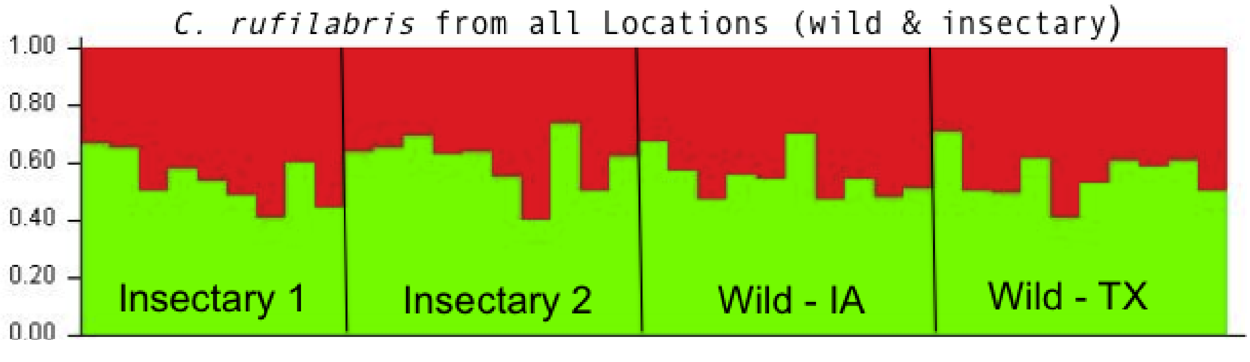

Figure 1. Bayesian Population assignment probabilities (y-axis) for *C. rufilabris* individuals (x-axis) collected from insectaries and from the wild using the recessive alleles model for dominant marker data in STRUCTURE 2.3.3. All individuals are assigned with relatively equal probability to both hypothetical populations (red and green) when two populations are assumed indicating a lack of population structure.

Bayesian clustering analysis performed in STRUCTURE v.2.3.3 revealed that there are two (K = 2) distinct genetic populations of *D. isaea* in the populations sampled (Figure 2). However, the structure found does not segregate by location as hypothesized.

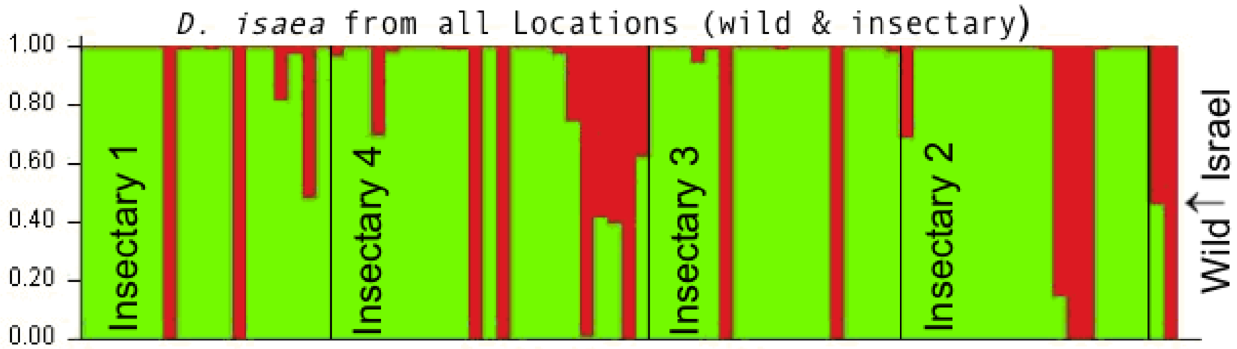

Figure 2. Bayesian population assignment probabilities (y-axis) for K = 2 populations of *D. isaea* individuals (x-axis) collected from 4 different insectaries and one wild population using the recessive alleles model for dominant marker data in STRUCTURE 2.3.3. Two genetically distinct populations (red and green) are indicated. However, no segregation by insectary or location is observed.

## Discussion

Our results did not support the hypothesis of reduced genetic variation among collections from insectaries versus field locations, nor did we find evidence of genetic variation among collections from insectaries. Heterozygosity values among *C. rufilabris* and among *D. isaea* from different locations were very similar. Patterns of polymorphic variation also repeated without substantial change among the insectary-reared and wild-caught *C. rufilabris* or *D. isaea*.

However, the partitioning of genetic variation differs between *C. rufilabris* and *D. isaea*. All *C. rufilabris* examined (*N*=40 individuals) belong to the same genetic group whereas within each *D. isaea* collection location wasps tend to belong to either group A (red) or group B (green). The mechanisms generating these patterns are largely unknown.

Our data seems to suggest that growers looking for a biological control agent to release in their crops could choose from any insectary without concern of choosing a misplaced locally adapted population. However, although the product seems to be uniform enough to allow reproduction, even among insects from producers across the world, field performance could still differ if specific genes involved in local adaptation differ among locations. Thus, fitness tests should be conducted among insects from different insectaries to discard this possiblity.

Future research should include execution of a larger study. This could include a greater array of biological control species to be surveyed, but more importantly a larger sample size of insectary and wild-caught individuals of these species should be analyzed to further support the trend seen thus far. In addition, assessing variation with another set of markers (e.g., microsatellites, SNPs, etc.) and linking genetic information to natural enemy fitness parameters will further strengthen our results.

## Acknowledgements

Species identifications were validated by Bob Wharton and John Oswald. Peter Krauter, Josephine Antwi and Kyle Harrison provided technical assistance. The research was supported by a National Science Foundation grant (#1062178 to KMH).

## References

Baker, D. A., H. D. Loxdale, and O. R. Edwards. 2003. Genetic variation and founder effects in the parasitoid wasp, *Diaeretiella rapae* (M'intosh) (Hymenoptera: Braconidae: Aphidiidae), affecting its potential as a biological control agent. Mol. Ecol. 12: 3303–3311.

Falush, D, M. Stephens, and J. K. Pritchard. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. Notes 7: 574–578.

Hartl, D. L., and A. G. Clark. 1997. Principles of Population Genetics, 3rd Ed. *Population Substructure*: F *Statistics* (119). Sinauer Associates, Inc., Sunderland, MA

Hopper, K. R., R. T. Roush, and W. Powell. 1993. Management of Genetics of Biological-Control Introductions. Ann. Rev. Entomol. 38: 27–51.

Pritchard, J. K., X. Wen, and D. Falush. 2010. Documentation for STRUCTURE software: version 2.3. http://pritch.bsd.uchicago.edu/structure.html.

Roderick, G. K., and M. Navajas. 2003. Genes in New Environments: Genetics and Evolution in Biological Control. Nat. Rev. Genet. 4: 889–899.

Vos P, R. Hogers, M. Bleeker, M. Reijans, T van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407–4414.

# Pesticide use declines and biological control increases in California greenhouses

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## Introduction

Excessive use of pesticides in the production of floricultural crops has been an issue for decades. While certainly not true when focusing on all growing operations, the industry as a whole is defined by its bad actors and the result is that the entire industry has a negative association with pesticide use. This seems to come to the fore and makes good press around Valentines Day[2]. Much of the focus on excess use of pesticides in cut flowers has been in South America (Stewart, 2007) and this has led to some harsh criticism of the industry (Donohue, 2008). However, there has been concern in California[3] as well, where approximately 80% of the cut flowers in the US are produced. The negative aspects usually focus on the health and safety or farm workers, and historically the floriculture industry does not have a good track record. For example, data from 2003[4] indicate that almost 30% of all the incidents involving farm workers and pesticide residue exposure in California occurred in ornamental crops and this ranked second among all agricultural crops produced in the state. Of course this information is close to 10 years old, so what is current situation? In reality, pesticide use is declining in California cut flowers and there is a broad adoption of augmentative biological control by this industry. This has been a gradual change over the past 30 years, but this can only be accurately tracked over the past 10 years. Here I review the decline in pesticide use and discuss the underlying reasons for this, focusing on the adoption of biological control.

## Trends in Pesticide Use In California

In 1990, California became the first state to require full reporting of agricultural pesticide use. Growers were required to turn in monthly Pesticide Use Reporting forms to their County Agricultural Commissioner. The result has been a massive database (compiled and maintained by the California Department of Pesticide Regulation [CA DPR]). This allowed a comprehensive look/analysis of overall pesticide use in the state and these data can be examined by commodity, by pesticide, by county, etc. There have been changes over the more than 20 years in terms of the commodity categories where these data have been collected. During the early years of data collection, ornamental production was not broken down into sub categories, so it was not possible to separate pesticide use in greenhouse cut flowers, for example, from pesticide use in field grown nursery stock. This made an analysis of pesticide use in ornamental production more difficult. However, this has changed and by 2001 the categories were more clearly defined. Therefore a comprehensive look at pesticide use in greenhouse cut flower production really has to start in 2001.

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[2] http://www.onegreenplanet.org/lifestyle/flowers-the-ugly-truth/

[3] http://abcnews.go.com/Health/Technology/story?id=118255&page=1#.UFjBjxgmYbc

[4] www.cdpr.ca.gov/docs/whs/**2003**pisp.htm

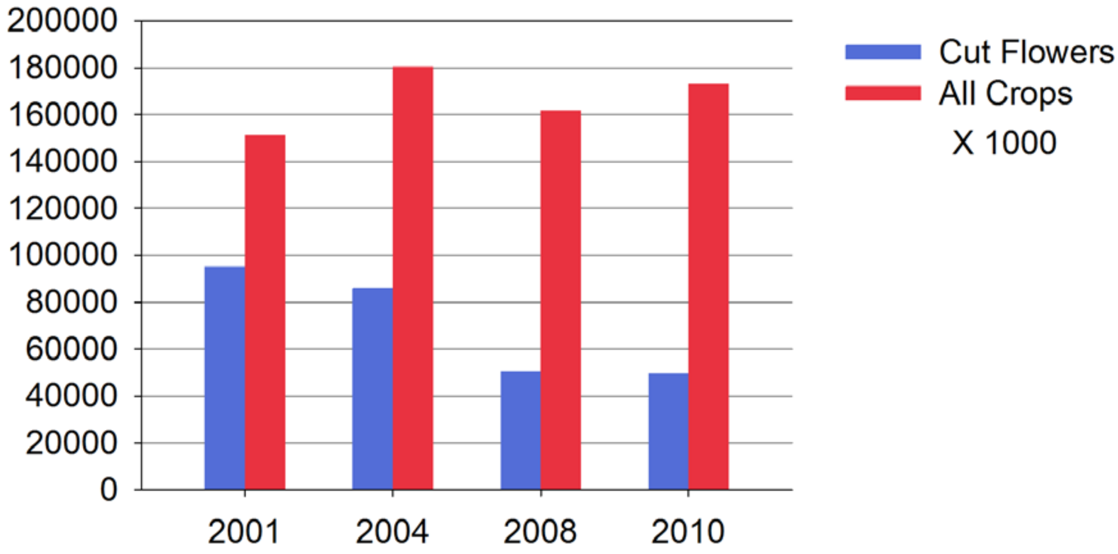

Figure 1. Pounds of pesticide applied in California on all crops (red bars) and on cut greenhouse cut flowers (blue bars) from 2001-2010. The amounts applied to all crops must be multiplied by 1000.

From 2006 to 2009, overall pesticide use in California dropped, but from 2009-2010 pesticide use increased by 9.5 % (15 million pounds active ingredient (AI.) [5] (Figure 1). In 2010, California used more than 173 million pounds of pesticide AI. Despite an increase in pesticide use across all of California crops in 2010, pesticide use in greenhouse cut flowers continued to decline. In 2001, cut flowers ranked 49th among all agricultural commodities in terms of total pesticide use. By 2010, this ranked had dropped to 82nd.

There has been a decline in cut flower acreage over time, but this reduction does not explain the 50% reduction in pesticide use that occurred from 2001 to 2010. In fact the data from these dates are remarkably similar. In 2001, 48,469 pesticide applications were made to 86,800 acres and in 2010, 45,894 applications were made to 81,695 acres. In addition, as one looks at the cut flower statistics in California (at least from 2006-2010), in 2006 the total value was $318 million and in 2010, this had dropped to $286 million. Although the industry has fallen in size and value, this does not account for the dramatic decrease in pesticide use during the same period. It is important to note that along with a reduction in pesticide use has come a reduction in the number of incidents reported with field workers and pesticides. For greenhouse cut flowers in 2009[6], this dropped to 2% of the total number of cases in California agriculture.

A simple reduction in pesticide use does not tell the full story. Altering the type of pesticides used may be even more important. As noted above, CA DPR recorded an increase in pesticide use in 2010, but much of that use was attributed to the increased use of sulfur for control of powdery mildew in crops such as grapes. Inorganic sulfur, although it is not benign environmentally, is approved for organic production and the increased used of such a pesticide takes some of the sting out of a negative scenario where pesticide use is increasing statewide. Likewise, the mix of pesticides used by floriculture producers is changing. It is slanting away from the use

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[5] http://www.cdpr.ca.gov/docs/pressrls/2011/111228.htm

[6] www.c**dpr**.**ca**.gov/docs/whs/**2009**pisp.htm

of traditional, broad-spectrum materials such as organophosphates and carbamates towards inorganic products like soaps and oils, botanicals, insect pathogens, and insect growth regulators.

Statistics on pesticide use can often be confounded by the activity of the pesticide itself. For example, older chlorinated hydrocarbon insecticides (like DDT) were applied at rates of 4 lbs. AI. per acre, while the pyrethroid insecticides (like permethrin) are applied at 0.1 lbs. AI. per acre. Therefore, simply switching from DDT to Permethrin would achieve the goal of using less pesticide AI per acre, when in fact you are making the equivalent (and in many cases more) pesticide applications per acre. The reverse also can be true. Application rates of sulfur are often many times that of conventional powdery mildew materials (something that is generally true for 'safer' insecticides), so switching to sulfur (often viewed as positive because of its organic certification) will increase the total amount of pesticide applied per acre. The bottom line is that when you switch from one pesticide to another you need to take into consideration the activity and application rates in order to really determine whether there has been a reduction in AI. per acre in addition to the number of applications per acre. Such an analysis goes beyond the scope of this article, but I believe there has been a net decrease in both for greenhouse cut flower production in California from 2001 – 2010.

## Reasons Underlying the Change: Adoption of Biological Control

Biological Control has been widely adopted by the cut flower industry and has been a consistent and effective alternative to pesticide application. Of course, this scenario is mutually reinforcing: as growers reduce pesticide application there is greater opportunity for biological control to be successful. The western flower thrips complex (Rugman-Jones, 2010) represent a major pest of floriculture crops in the state so it follows that natural enemies used against this pest are among the most common natural enemies purchased by growers. These include *Amblyseius (Neoseiulus) cucumeris* Oudemans, *A. (Typhlodromips) swirksii* (Mesostigmata: Phytoseiidae) and *Hypoaspis (Stratiolaelaps) miles* (Berlese) (Mesostigmata: Laelapidae). Other widely used natural enemies include *Phytoseiulus persimilis* (Athias Henriot) and *Amblyseius (Neoseiulus) californicus* (McGregor) (Mesostigmata: Phytoseiidae) for control of spider mites and the parasitoid *Diglyphus isaea* Walker (Hymenoptera: Eulphidae) for control of the leafminer, *Liriomyza trifolii* (Burgress) (Diptera: Agromyzidae).

There are other factors that have reduced pesticide use and have allowed (or in some cases driven) growers to adopt biological control. These include: 1) the advent of 3rd party certification organizations that ultimately lead to pesticide reduction and a wiser choice of pesticides, 2) the greater use of screening in many greenhouses in the state, 3) the ever increasing rules and regulations focusing on groundwater and farm worker health and safety, 4) the greater acceptance of thresholds, where some insects can be tolerated on a crop before a pesticide is applied, 5) greater use of monitoring with light traps, pheromone traps and yellow/blue sticky cards, 6) increased frequency of spot spraying to control early pest infestations without treating the entire greenhouse, 7) the advent of effective 'reduced risk' materials that can replace traditional chemistry and offer the potential of compatibility with natural enemies, 8) a new generation of growers who have grown up under the concept of going green, and 9) a consuming public with a greater acceptance of organic production and with an increasing negative perspective of pesticides.

## Room to Improve

As noted earlier, pesticide use has declined in cut flower production in California by almost 50% from 2001 to 2010. However, from 2008 to 2010, there was only a slight decline (50,105 lbs. to 49,250 lbs., respectively). In 2009 there was a slight increase up to 51,000 lbs. – this suggests a trend where pesticide reduction has leveled off. The challenge is to keep this total pesticide use declining through the greater adoption of biological control. This is one of the goals of my laboratory and some of the strategies to do accomplish this will be presented.

## References

Donohue, M. 2008. Flowers, Diamonds and Gold: The Destructive Public Health, Human Rights, and Environmental Consequences of Symbols of Love. Hum. Rights Quart. 30: 164–182.

Rugman-Jones, P. F., M. S. Hoddle, and R. Stouthamer. 2010. Nuclear-mitochondrial barcoding exposes the global pest western flower thrips (Thysanoptera: Thripidae) as two sympatric cryptic species in its native California. J. Econ. Entomol. 103: 877–886.

Steward, A. 2007. Flower Confidential. Algonquin Books of Chapel Hill, Chapel Hill, NC.

# Session 8: Augmentative and Conservation Biological Control Techniques in Forestry

## Production and use of heteropteran predators for biological control of *Eucalyptus* pests in Brazil

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Asopinae predators of *Eucalyptus* spp. (Myrtaceae) pests in Brazil include *Brontocoris tabidus* Signoret, *Podisus distinctus* Stäl, *Podisus nigrispinus* Dallas and *Supputius cincticeps* Stäl (Heteroptera: Pentatomidae) (Figure. 1). These insects feed on larvae and pupae of Coleoptera and Lepidoptera defoliators and they can reduce populations of nymphs and adults of *Thaumastocoris peregrinus* Dellape & Carpintero (Hemiptera: Thaumastocoridae) (Souza et al. 2012).

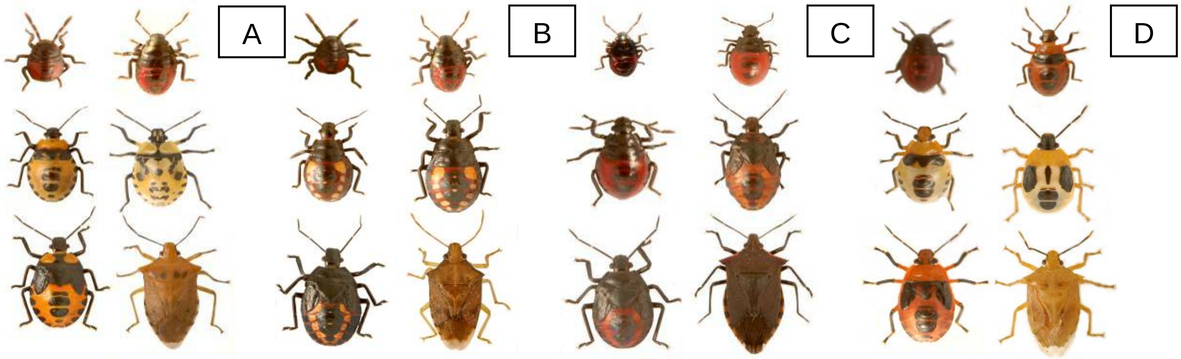

Figure 1. Nymphs of first, second, third, fourth and fifth instars and adults of *Brontocoris tabidus* (A), *Podisus nigrispinus* (B), *Podisus distinctus* (C) and *Supputius cincticeps* (D) (Heteroptera: Pentatomidae).

Asopinae predators reared in wooden cages (30 cm width × 30 cm length × 30 cm height) with wood screen on the upper and lateral sides and the front closed with a glass door. The cage bottom is lined with paper to facilitate cleaning. Branches of *Eucalyptus* spp. placed in the cages for *S. cincticeps* because this species needs plants. Asopinae predators can also be reared in the field in organza bags involving branches of *Eucalyptus* spp. or *Psidium guajava* L. (Myrtaceae). Egg masses of these insects removed daily from the cages and/or bags and maintained in Petri dishes until nymphs reach second instar (Figure 2).

Figure 2. A) Petri dish with eggs on pieces of cotton; B) wooden cages with branches of *Eucalyptus grandis* W.Hill ex Maiden (Myrtaceae) and C) organza bags involving branches of this plant in the field to rear Asopinae predators.

Food is provided according to rearing need to reduce cannibalism. Preys are placed on top and bottom of rearing cages with moistened cotton as the water source. Alternative prey such as pupae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), *Musca domestica* L. (Diptera: Muscidae) or *Bombyx mori* L. (Lepidoptera: Bombycidae) larvae (Figure 3) or natural prey such as *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), *Thyrinteina arnobia* Stoll and *Thyrinteina leucocerae* Rindge (Lepidoptera: Geometridae) caterpillars may be provided to predator nymphs in cages and/or rearing bags. The development period of *P. nigrispinus* nymphs was similar when fed *T. molitor* (T1) pupae or *M. domestica* larvae (T2) or to both prey simultaneously (T3) or on alternate days (T4) with average viability of 75%. Females were heavier in the T1 and T4, although with similar reproductive rates. Females of these predators produced the highest number of eggs and nymphs with both prey simultaneously (Zanuncio et al. 2001).

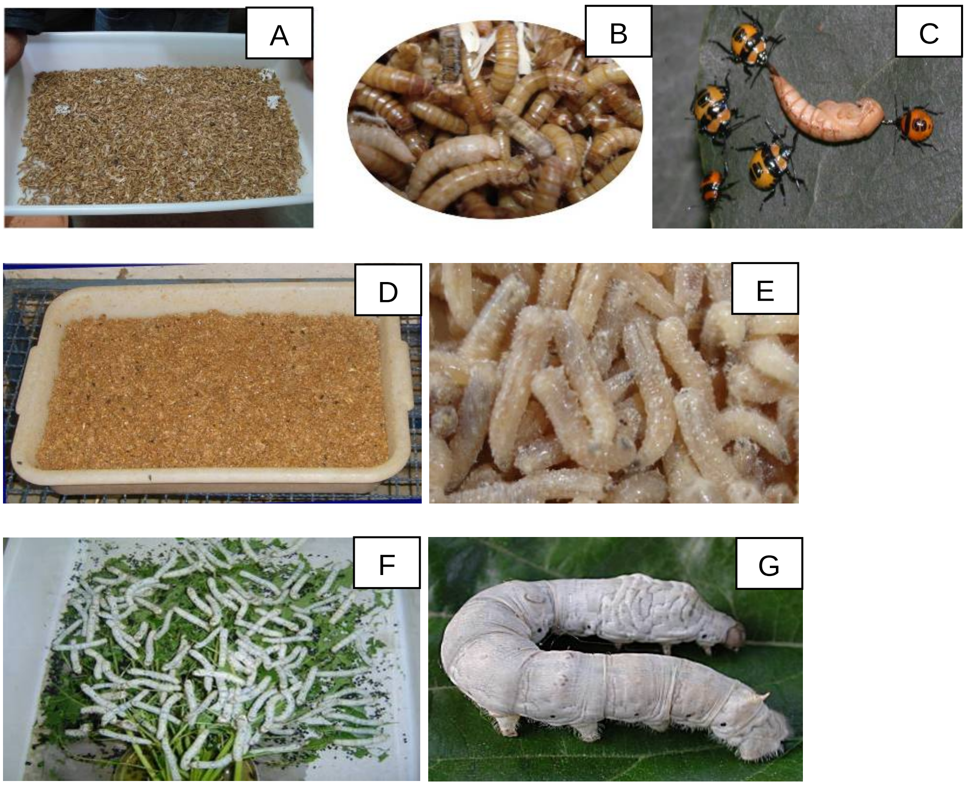

Figure 3. A) Plastic tray with larvae. B) Pupae of *Tenebrio molitor*. C) *Brontocoris tabidus* nymphs preying on larvae of this prey. D) Plastic tray with E) *Musca domestica* larvae (E). F) Plastic tray with branches of *Morus* sp. as a substrate to feed G) *Bombyx mori* caterpillars (G).

*Tenebrio molitor* can be reared in plastic trays of different sizes with 95% wheat bran (high carbohydrate) and 5% beer yeast (rich in proteins). Slices of *Sechium edule* (Jacq.) Swartz (Cucurbitaceae) and *Saccharum* spp. (Poaceae) are placed on the substrate as a moisture source and a paper towel cover to reduce brightness.

Three hundred grams of *M. domestica* pupae are needed to start a rearing facility with a capacity of 10,000 flies. This material can yield 2.5 to 3 kg of larvae per day with young adult flies fed with milk and sugar. A tray with moistened wheat bran with water and milk is placed inside the nursery as an oviposition site. The tray with the substrate with postures is removed on the next day and its contents placed in a rearing tray with slightly moistened wheat bran. The larvae can be transferred with a trowel on the next day and offered to the predators.

Lepidoptera defoliators can be controlled at egg and larva stages in the field by nymphs and adults of Asopinae predators. Pupae of these pests can also be controlled by nymph and adult predators (Figure 4) and by parasitoids such as *Palmistichus elaeisis* Delvare & LaSalle and *Trichospilus diatraeae* Margabandhu & Cherian (Hymenoptera: Eulophidae). *Brontocoris tabidus* is the first to arrive in areas infested by defoliating caterpillars, followed by the other predator species, especially *P. nigrispinus*. These natural enemies build large populations when egg parasitoids start to reduce their populations. Barriers sprayed with *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (Polanczyk et al. 2009) are erected around outbreaks of pests to reduce its spread.

The diversity of natural enemies is an alternative in Integrated Pest Management (IPM). The emergence and the cycle from egg to adult of *P. elaeisis* parasitizing pupae of *T. molitor* predated by *P. nigrispinus* for 48 hours showed lower values compared to 24 hours, but female parasitoids that emerged had longer lifespans. Predation of pupae parasitized by *P. elaeisis* reduced emergence and sex ratio, but increased longevity of this parasitoid, which may reduce its populations in the field. Moreover, shorter immature stages and increased longevity of *P. elaeisis* could benefit the impact of this parasitoid in the field with the predator *P. nigrispinus*.

Figure 4. A) Asopinae predators feeding on pupae, B) caterpillar, and C) eggs of Lepidoptera defoliators.

Vallourec & Mannesmann Forest Ltda. is a pioneer company in Brazil using biological control for pest management in *Eucalyptus* spp. plantations. The first laboratory of biological control was built at the Center for Support of Research and Development in Forest (CAPEF) in Paraopeba, Minas Gerais State, Brazil in 1989 to mass produce and release *P. nigrispinus* to control outbreaks of *Apatelodes sericea* Schaus (Lepidoptera: Eupterotidae), *Glena* spp. (Lepidoptera: Geometridae), *Psorocampa denticulata* Schaus (Lepidoptera: Notodontidae) and *T. arnobia*, besides other lepidopteran species. This is important because yield losses between 5% and 10% were recorded for trees with 75% and 100% defoliation, respectively, and therefore pest control is important to maintain wood productivity. Biological control is an effective and sustainable pest management option because it reduces population levels of pests and the number of chemical applications.

*Podisus nigrispinus* is preferable for mass rearing and pest control in *Eucalyptus* spp. plantations because it presents a well-developed and efficient technique compared to other predatory Asopinae, besides producing more eggs per female with higher predation rates, shorter life cycle and it can feed on artificial diet during periods of prey shortage. Vallourec & Mannesmann Forestry Ltda. has two laboratories to mass rear *M. domestica* with an average annual production of 300,000 predators (Figure 5). Environmental conditions affect the duration of the life cycle of predators and, therefore, temperature, relative humidity and photoperiod should be controlled.

*Podisus nigrispinus* has been released with or without pests in the field. In the first case, predators released after detection of the pest by monitoring, which is the first major step in IPM. This predator is efficient when the pest population level is below the economic injury level, i.e., nine caterpillars/100 leaves of *Eucalyptus* spp. Releases were made in outbreaks every 10m. *Podisus nigrispinus* usually prey on 4 to 5, 1st and 2nd instar larvae or 2 to 3, 4th and 5th instar caterpillars. A total of 1,500 ha of *Eucalyptus* spp. with outbreak of the defoliating caterpillars *Thyrinteina* spp. and *Glena* spp. was controlled with *P. nigrispinus* in the first three months of 2012. It is necessary to mention that this control prevented an increase in the area damaged by defoliating caterpillars to adjacent eucalyptus plantations.

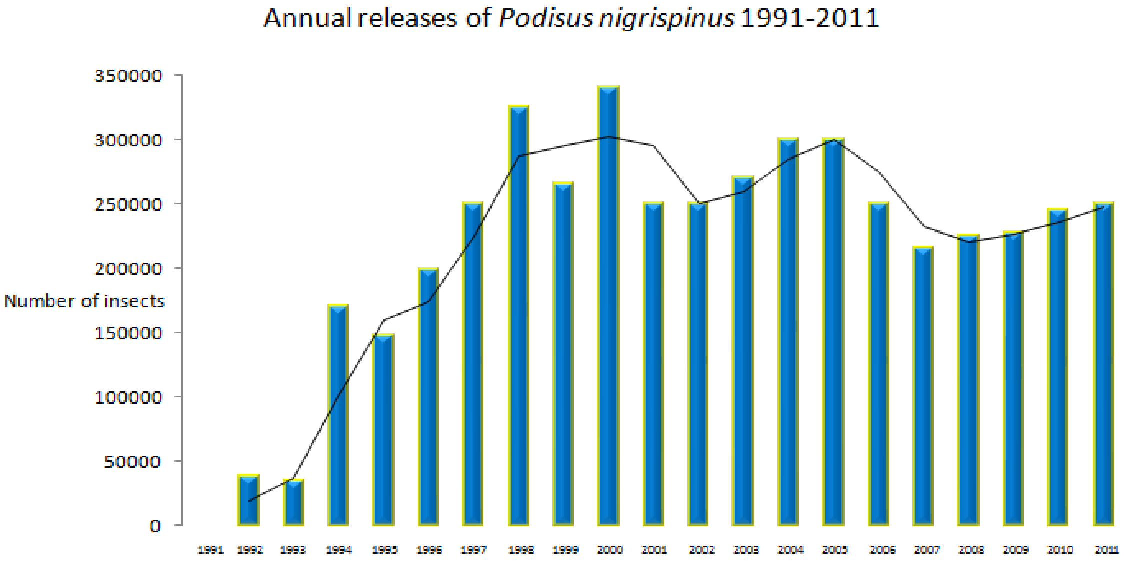

Figure 5. Number of individuals of *Podisus nigrispinus* (Heteroptera: Pentatomidae) released from 1991 to 2011 in *Eucalyptus* spp. (Myrtaceae) plantations of the company Vallourec & Mannesmann Forest Ltda in Minas Gerais State, Brazil.

## Acknowledgements

To “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)”, “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)” and

“Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG)” for financial support.

## References

Polanczyk, R. A, J. C. Zanuncio, and S. B. Alves. 2009. Relationship between chemical properties of the soil and the occurrence of *Bacillus thuringiensis*. Ciência Rural 39: 1–5.

Souza, G. K., T. G. Pikart, F. C. Pikart, J. E. Serrão, C. F. Wilcken, and J. C. Zanuncio. 2012. First record of a native heteropteran preying on the introduced pest, *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae), in Brazil. Florida Entomol. 95: 517–520.

Zanuncio, J. C., A. J. Molina-Rugama, J. E. Serrão, and D. Pratissoli. 2001. Nymphal development and reproduction of *Podisus nigrispinus* (Heteroptera: Pentatomidae) fed with combinations of *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae and *Musca domestica* (Diptera: Muscidae) larvae. Biocon. Sci. Technol. 11: 331–337.

# Biological control of forest pests by insect natural enemies in China

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In the last 20 years, China has experienced severe attacks by many forest pests. Several of these pests have been the target of biological control programs against these pests by using insect natural enemies. These programs are summarized here.

Native to North America, the fall webworm, *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae), has been sustainably controlled by an effective pupa gregarious endoparasitoid, *Chouioia cunea* Yang (Hymenoptera: Eulophidae) (Yang,1989), which is native to China, and HcNPV virus by spraying the virus in the larval stage and releasing the parasitoid in its pupal stage (Duan et al. 2009; Yang et al. 2005b; Yang et al. 2006b; Yang and Zhang 2007; Yang et al. 2008b).

The red turpentine beetle, *Dendroctonus valens* (Coleoptera: Scolytidae), also native to North America, was suppressed by a predator, *Rhizophagus grandis* (Coleoptera: Rhizophagidae) introduced from Belgium. Mass rearing and field-release techniques were developed and a total of 3,334 ha of pine forests (*Pinus tabulaeformis*) were effectively protected (Zhao 2006; Zhao et al. 2008) .

Pine wilt disease is right now the most destructive forest pest in China. One of the strategies to control the nematode focuses on its vector, *Monochamus alternatus* (Coleoptera: Cerambycidae). We found two dominant parasitoids by investigating infestation areas in China*, Dastarcus helophoroides* (Fairmaire) (Coleoptera: Bothrideridae) parasitizing in medium and late instar larvae and pupae of the cerambycid, and *Sclerodermus* sp. (Hymenoptera: Bethylidae) parasitoid of young larval stage. Then, mass rearing and release techniques were studied and developed. By releasing the two parasitoids, 92.6% of the cerambycid were parasitized in the first year of the experiments. This, combined with the setting up of lure trees and black light traps developed by us, resulted in the control of the pine wilt disease (Zhang and Yang 2006; Lai et al. 2012; Yang et al. 2012c; Yang et al. 2013).

The oak longhorn beetle, *Massicus raddei* (Coleoptera: Cerambycidae), is a serious pest of oak forests in northeastern China. A special black light trap was developed for attracting adult beetles. In 2008 and 2011, 24.15 and 14.9 tons of the cerambycid adults were attracted and killed by the black light trap in Jilin Province (Yang et al. 2011b). A parasitoid of young larvae, *Sclerodermus pupariae* Yang *et* Yao (Hymenoptera: Bethylidae), was released to control the 2nd and 3rd instar larvae of the beetle, with parasitism rates of 46% (Jiang et al. 2010; Wang et al. 2010b;Yang et al. 2012b; Wu et al. 2008). A biotype of the parasitoid *Dastarcus helophoroides* (Coleoptera: Bothrideridae) was found to be an effective parasitoid of medium and mature instars in natural populations and was also used as a bocontrol agent to control the pest, with parasitism rates over 90% (Yang et al. 2012a). The cerambycid was effectively controlled by applying the above integrated techniques (Tang et al. 2012).

The Asian longhorn beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae), is a severe pest of many broadleaved tree species in China. The popular shelter belts of “green great wall” in “three North” of China were destroyed in 1980~1995 by this insect. We have developed a biological control technique based on the release of the parasitoid *Sclerodermus guani* (Hymenoptera: Bethylidae) on host young larval stages

(1st and 3rd instars), with parasitism rates of 35.6% (Yao and Yang 2008), and of a biotype of *Dastarcus helophoroides,* parasitizing mid- and late instar larvae and pupae of *A. glabripennis* naturally, with parasitism rates of 92.2% on average. The longhorn beetle was successively controlled in experimental forests in Xinjiang and Inner Mongolia (Li et al. 2009a).

The emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), is native to eastern Asia, including China. In North America, it has become a very serious pest of on ash trees. Its natural enemies were investigated in China and seven species were found. *Spathius agrili* Yang (Hymenoptera: Braconidae) (Yang et al. 2005a), *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophiae) (Yang et al. 2006a), *Oobius agrili* Zhang *et* Huang (Hymenoptera: Encyrtidae) (Zhang et al. 2005) and *Sclerodermus pupariae* Yang *et* Yao (Hymenoptera: Bethylidae) (Yang et al. 2012b) dominate the parasitoid complex and have high potential for biological control of the pest in North America. The biology, behavior, ecology and mass rearing techniques of the parasitoids were studied (Wang et al. 2006; Wang et al. 2008; Wang et al. 2010a; Yang et al. 2008a; Yang et al. 2010). Right now, the first three parasitoids have been introduced to USA and *S. agrili* has established in at least five US States.

Another two species of longhorn beetles, *Batocera lineanata* (formerly *Batocera horsfieldi* in China) attacking poplar trees in Yangtze River valley and middle China, and *Apriona germarii* attacking *Sophora japonica* (Leguminosae)*,* a main ornamental tree species in China, were controlled by releasing their own biotypes of *Dastarcus helophoroides,* parasitoid of later instar larvae and pupae, with parasitism rates of 92.5% on average (Li et al. 2009b; Lu et al. 2011; Wang et al. 2013; Yang et al. 2011a).

## References

Duan, Y. L., L. J. Qu, Y. Z. Wang, Y. A. Zhang, W. Q. Tao, L. Guan, and Z. Q. Yang. 2009. Sustainable effects for *Hyphantria cunea* Nuclear Polyhedrosis Virus on the host insects and the diffusion approach of the virus. Scientia Silvae Sinicae 45(6): 83–86, 62.

Jiang, J., Z. Q. Yang, Y. L. Tang, H. Tang, G. J. Sun, and Z. Q. Gao. 2010. Trapping technology on adults of *Massicus raddei* by a special black light. J. Environ. Entomol. 32(3): 369–374.

Lai Y.X., Y. H. Wang, X. Y. Wang, Z. Q. Yang, Y. L. Tang, R. H. Qin, Y. N. Zhang, and Y. F. Zhang. 2012. A field test of controlling the pine wilt disease by technique of isolating *Monochamus alternatus* beetle on forest spot and releasing parasitoid *Sclerodermus guani* (Hymenoptera: Bethylidae). Chinese J. Biol. Contr. 28(4): 460–465.

Li M. L., Y. Z. Li, Q. Lei, and Z. Q. Yang. 2009a. Biocontrol of Asian longhorned beetle larva by releasing eggs of *Dastarcus helophoroides* (Coleoptera: Bothrideridae). Scientia Silvae Sinicae 45(4): 78-82.

Li J. Q., Z. Q. Yang, Y. L. Zhang, Z. X. Mei, Y. R. Zhang, and X. Y. Wang. 2009b. Biological control of *Batocera horsfieldi* (Coleoptera: Cerambycidae) by releasing its parasitoid *Dastarcus helophoroides* (Coleoptera: Bothrideridae). Scientia Silvae Sinicae 45(9): 94-100.

Lu X., Z. Q. Yang, X. Sunn, L. Qiao, X. H. Wang, and J. R. Wei. 2011. Biological control of *Apriona swainsoni* (Coleoptera: Cerambycidae) by releasing the parasitic beetle *Dastarcus helophoroides* (Coleoptera: Bothrideridae). Scientia Silvae Sinicae 47(10): 116-121.

Tang, Y. L., Z. Q. Yang, X. Y. Wang, H. Tang, J. Jiang, K. Wei, and J. Lü. 2012. Biocontrol of oak longhorn beetle, *Massicus raddei* by releasing parasitoid *Dastarcus helophoroides* (Coleoptera: Bothrideridae) adults and eggs. Scientia Silvae Sinicae 48(7): 186–191.

Wang X. H., Z. Q. Yang, Y. L. Zhang, and Y. L. Tang. 2013. Biological control of *Apriona swainsoni* (Coleoptera: Cerambycidae) by releasing three species of parasitoids. Acta Entomologica Sinica, 56(1) (In press).

Wang, X. Y., Z. Q. Yang, G. J. Liu, and E. S. Liu. 2006. Relationships between the emergence and oviposition of ectoparasitoid *Spathius agrili* Yang and its host emerald ash borer, *Agrilus planipennis* Fairmaire. Acta Ecologica Sinica 26 (4): 1103–1109.

Wang, X. Y., Z. Q. Yang, W. Hui, and J. R. Gould. 2008. Effects of host size on the sex ratio, clutch size, and size of adult Spathius agrili, an ectoparasitoid of emerald ash borer. Biol. Control. 44: 7-12.

Wang, X. Y., Z. Q. Yang, J. R. Gould, H. Wu, and J. H. Ma. 2010a. Host-seeking behavior and parasitism by *Spathius agrili* Yang (Hymenoptera: Braconidae), a parasitoid of the emerald ash borer. Biol. Control 52: 24–29.

Wang, X.Y., Z. Q. Yang Y. L. Tang, J. Jiang, C. Gao, and Y. C. Liu. 2010b. Parasitism of *Sclerodermus pupariae* (Hymenoptera: Bethylidae) on the young larvae of *Massicus raddei* (Coleoptera: Cerambycidae). Acta Entomologica Sinica 53(6): 675–682.

Wu, H., X. Y. Wang, M. L. Li, Z. Q. Yang., F. X. Zeng, H.Y. Wang, L. Bai, S. J. Liu, and J. Sun. 2008. Biology and mass rearing of *Sclerodermus pupariae* Yang *et* Yao (Hymenoptera: Bethylidae), an important ectoparasitoid of the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae) in China. Acta Entomologica Sinica 51(1): 46–54.

Yang, Y. L., Z. Q. Yang. and X. Y. Wang. 2013. Predation and parasitism of *Sclerodermus* sp. (Hymenoptera: Bethylidae) on the young larvae of *Monochamus alternatus* (Coleoptera: Cerambycidae). Scientia Silvae Sinicae 48 (in press)

Yang, Z. Q. 1989. A new genus and species of Eulophidae (Hymenoptera: Chalcidoidea) parasitizing *Hyphantria cunea* (Drury) (Lepidoptera: Arctidae) in China. Entomotaxonomia 11(1–2): 117–130.

Yang, Z. Q. and Y. A. Zhang. 2007. Researches on techniques for biocontrol of the fall webworm, *Hyphantria cunea,* a severe invasive insect pest to China. Chinese Bull. Entomol. 44: 465–471.

Yang, Z. Q., J. S. Strazanac, P. M. Marsh, C. van Achterberg and W. Y. Choui. 2005a. First recorded parasitoid from China of *Agrilus planipennis*: a new species of *Spathius* (Hymenoptera: Braconidae: Doryctinae). Ann. Entomol. Soc. Am. 98: 636–642.

Yang, Z. Q., X. Y. Wang, C. Z. Wang, X. R. Qiao, and J. J. Pang. 2005b. Studies on Utilizing parasitoid *Chouioia cunea* Yang (Hymenoptera: Eulophidae) for sustainable control of fall webworm. Scientia Silvae Sincae 41(5): 72–80.

Yang, Z.Q., J. S. Strazanac, Y. X. Yao, and X. Y. Wang. 2006a. A new species of emerald ash borer parasitoid from China belonging to the genus *Tetrastichus* Haliday (Hymenoptera: Eulophidae). Proc. Entomol. Soc. Washington 108: 550–558.

Yang, Z. Q., J. R. Wei, and X. Y. Wang. 2006b. Mass rearing and augmentative releases of the native parasitoid *Chouioia cunea* (Hymenoptera: Eulophidae) for biological control of the introduced fall webworm *Hyphantria cunea* in China. BioControl. 51: 401–418.

Yang, Z. Q., X. Y. Wang, J. R. Gould, and H. Wu. 2008a. Host specificity of *Spathius agrili* Yang (Hymenoptera: Braconidae), an important parasitoid of the emerald ash borer. Biol. Control 47: 216–221.

Yang, Z. Q., X. Y. Wang, J. R. Wei, H. R. Qu, and X. R. Qiao. 2008b. Survey of the native insect natural enemies of *Hyphantria cunea* (Drury) (Lepidoptera: Arctiidae) in China. Bull. Entomol. Res. 98: 293–302.

Yang, Z. Q., X. Y. Wang, R. G. Juli, C. R. Richard, Y. N. Zhang, G. J. Liu, and E. S.Liu. 2010. Biology and behavior of *Spathius agrili*, a parasitoid of the emerald ash borer, *Agrilus planipennis*, in China. J. Insect Sci. 10(: 1–13.

Yang, Z. Q., J. Q. Li, Z. X. Mei, X. Y. Wang, Z. G. Jin, and X. D. Meng. 2011a. Biocontrol of *Batocera horsfieldi* (Coleoptera:Cerambycidae) attacking ash trees by releasing the parasitoid *Dastarcus helophoroides* (Coleoptera: Bothrideridae). Scientia Silvae Sinicae 47(12): 78-84.

Yang, Z. Q., X. Y. Wang, B. Wang, Y. P. Yu, Z. M. Dong, Z. H. Mou, and H. S. Wang. 2011b. Relationship between adult emergence of *Massicus raddei* (Coleoptera: Cerambycidae) and temperature and relative humidity. Acta Ecologia Sinica 31(24): 7486–7491.

Yang, Z. Q., Y. L. Tang, J. Jiang, X. Y. Wang, H. Tang, J. Lü, and Y. Gao. 2012a. Study on alternative hosts of *Dastarcus helophoroides*, a parasitoid of *Massicus raddei* (Coleoptera: Cerambycidae), and its population-keeping mechanism in oak forest. Acta Ecologia Sinica 32(24) (in press).

Yang, Z. Q., X. Y. Wang, Y. X. Yao, and L. M. Cao. 2012b. A new species of *Sclerodermus* Latreille (Hymenoptera: Bethylidae) parasitizing *Agrilus planipennis* (Coleoptera: Buprestidae) from China with a key to Chinese species in the genus. Ann. Entomol. S. Am. 105: 619–627.

Yang, Z. Q., X. Y. Wang, Y. N. Zhang, C. N. Situ, J. Wang, and F. Y. Fu. 2012c. Control effects of the pine wood nematode disease transmitted by *Monochamus alternatus* through releasing parasitoid *Dastarcus helophoroides* (Fairmaire) and using bait-trees. Chinese J. Biol. Contr. 28(4): 490–495.

Yao, W. J., and Z. Q. Yang. 2008. Studies on biological control of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) with a parasitoid, *Sclerodermus guani* (Hymenoptera: Bethylidae). J. Environ. Entomol. 30: 127-134.

Zhang, Y. N., and Z. Q. Yang. 2006. Studies on the natural enemies and biocontrol of *Monochamus alternatus* Hope (Coleoptera: Cerambycidae). Plant Prot. 32: 9–14.

Zhang, Y. Z., D. W. Huang, T. H. Zhao, H. P. Liu, and L. S. Bauer. 2005. Two new species of egg parasitoids (Hymenoptera: Encyrtidae) of wood-boring beetle pests from China. Phytoparasitica. 33: 253–260.

Zhao, J. X. 2006. Biocontrol of *Dendroctonus valens* in China. Dissertation of PH.D., Chinese Academy of Forestry, Beijing, China.

Zhao, J. X., Z. Q. Yang, G. W. Li, and J. R. Wei. 2008. Studies on the major factors of mass rearing of *Rhizophagus grandis* and their controlling methods. J. Environ. Entomol. 30: 336–343.

# Perspectives and challenges to biological control of red gum lerp psyllid

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The red gum lerp psyllid (RGLP), *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae), was detected in Mogi-Guassu, State of São Paulo, Brazil, in June, 2003 (Wilcken et al. 2003) and nowadays are present in 14 Brazilian states and 7 countries of South America. The pest causes sooty mold and tip defoliation of trees, reaching total defoliation after some months of infestation. In *Eucalyptus camaldulensis* plantations of Northern São Paulo State, 40 to 95 % of tree mortality was observed, after some years of high infestation.

The feasible control options are selection of resistant eucalyptus clones to RGLP and biological control. The results of the first method are slow, considering the research period and the time to change the genetic material in field. Biological control can be effective in shorter time than plant resistance development.

Parasitoids importation from Australia was done in the USA and only one species, *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) established in California (Dahlsten et al. 2003).

In Brazil, the presence of some native natural enemies, as entomopathogenic fungi, lady beetles, green lacewings and hoverflies was investigated. However these organisms are considered not effective to regulate de RGLP population (Dahlsten et al. 2003). In 2003, the parasitoid *P. bliteus* was detected in Piracicaba, SP, Brazil (Berti Filho et al. 2003). After this, a rearing of this parasitoid was initiated in laboratory conditions, to release in infested regions of Brazil. Meanwhile, importations of *P. bliteus* populations wew made from Mexico, to increase the parasitoid genetic variability and adaptation capacity to Brazilian conditions.

Life parameters of *P. bliteus* vary geographically. In California (USA) *P. bliteus* females laid 125.7 eggs/female, meaning 125.7 nymphs parasitized, with 88.1 % of eggs deposited in the first 22 days of its lifespan. Adult longevity varied from 40.8 days at 17°C to 14.2 days at 32°C and the immature developmental time was 22.6 days at 22°C and 12.6 days at 30°C. Oviposition preference was to 3rd and 4th instars of *G. brimblecombei*, with 85.1 % of eggs laid in these two instars (Daane et al. 2005). In Mexico, the reproduction rate of the parasitoid was 17 adults produced per *P. bliteus* female and the immature development period was 41.6 days at 16.1°C and 16.3 days at 20.9°C (Plascencia-Gonzalez et al. 2005).

The parasitoid *P. bliteus* has been maintained in laboratory rearing, in cages with *E. grandis x E. camaldulensis* clone seedlings, producing approximately 100 parasitoids/cage/month. The parasitoids produced were released in five Brazilian states and are now present in all areas infested by RGLP. Biological control has been effective in Coastal zones and Southern region until South of SP. In Central region of Brazil (Cerrado region), and part of Northeastern region, the pest infestations have been high during the drier season (July to November), when the parasitism rate is too low. The main constraints of this parasitoid are: 1) occurrence of arrenothokous parthenogenesis in lab and field conditions; 2) preference by specific nymph instars (85 % of parasitism to 3rd and 4th nymph instars; 3) occurrence of superparasitism (reduction in number of fertile females per cage) (Daane et al. 2005); 4) environmental conditions distinct between pest and parasitoid (*G. brimblecombei*: best temperature: 26°C; best RH: bellow 50-40% (dry conditions) (Firmino-Winckler et al. 2009), *P. bliteus*: best temperatures: 29 to 30°C; RH: above ± 60%). The effect of higher temperatures in inland areas comparing with coastal areas in California was suggested to explain the reduced parasitism by *P. bliteus* in inland areas (Erbilgin et al. 2004; Sime et al. 2004).

As ther was little information concerning continuous releases of this parasitoid in Eucalyptus plantations, an experiment was carried out in a block with 19 ha of *Eucalyptus camaldulensis* plantation (6.5 years old), naturally highly infested with red gum lerp psyllid since 2003. Systematic parasitoid releases (20 couples of parasitoid/point) were made during the dry season (May to October 2006-2007) in five points (replications) in an *E. camaldulensis* plantation in State of Sao Paulo. Natural parasitism (control treatment) of the same parasitoid species was done in five other replications. The adults of *P. bliteus* used were reared in laboratory conditions (Wilcken et al. 2010). Eight releases were made between May and September 2006 (4 releases) and 2007 (4 releases) at intervals of approximately 20 days. The evaluations consisted in removing a branch of a tree in each point, taking 10 leaves/branch casually, and counting the total number of *G. brimblecombei* nymphs (3rd to 5th instars) and mummified nymphs per leaf. The data was analyzed with an ANOVA and the means compared by Tukey's test.

In area where releases were carried out, the parasitism level averaged 45.9% and 49.1%, during 2006 and 2007 respectively, while in area without releases, the natural parasitism level averaged 3.5%, in both years (Figure 1) (Ferreira Filho 2010). Our results show that it is possible to maintain an important pest control level with systematic releases during the more susceptible period to the pest (four months) in field conditions. However, *P. bliteus* apparently showed low dispersion capacity, because the check area was located at a distance of approximately 450 m, and parasitism levels were very low during the two years.

*Atopozelus opsimus* (Hemiptera: Reduviidae), a predatory bug that prey on nymphs and adults of *G. brimblecombei*, was also found in Brazil, mainly in dry season (Dias et al. 2012). This predator can prey up to 7 adults and 6 nymphs of different instars of RGLP in 36 h in laboratory conditions (Dias et al. 2012). This species can prey on all nymphal instars and adults, and it occurs naturally during the dry period. More studies are necessary to evaluate this predator in controlled and field conditions to determine the real pest control potential.

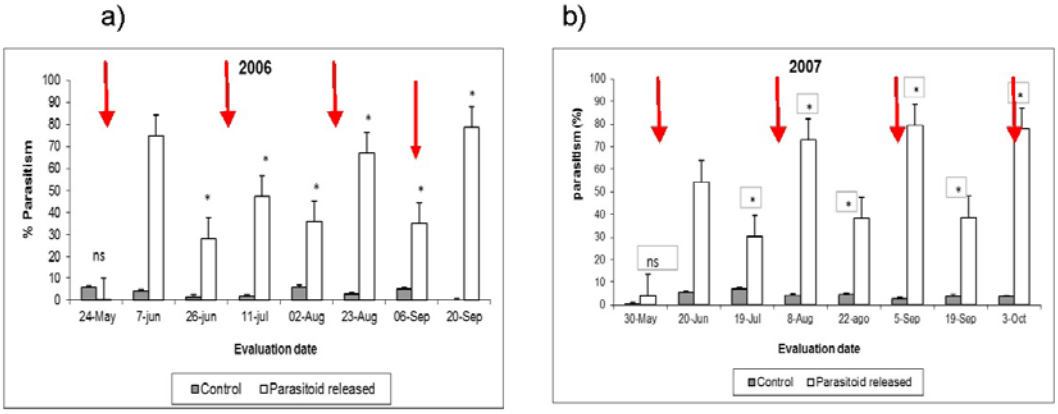

Figure 1. Parasitism by *Psyllaephagus bliteus* of *Glycaspis brimblecombei* nymphs after parasitoid releases in *Eucalyptus camaldulensis* plantation. Luis Antônio, SP, Brazil, a) May-Sep, 2006 and b) May-Oct, 2007. Obs.: Red arrows: Date of parasitoid releases. * Significant at p ≤ 0.01; ns: non-significant.

Entomopathogenic fungi are another tactics that were considered to manage against RGLP nymphs. In greenhouse experiment, commercial formulations of three different fungi species were tested: 1) *Beauveria bassiana*, 2) *Metarhizium anisopliae,* and 3) *Lecanicillium longisporum*. The higher doses of two formulations *B. bassiana* and *M. anisopliae* caused high mortality rates (> 80 %) showing potential to use these biological insecticides in *G. brimblecombei* control (Pogetto et al. 2011). Further studies are necessary to evaluate the compatibility or not of these entomopathogenic fungi to parasitoid *P. bliteus*.

Although *P. bliteus* are established in Eucalyptus plantations in Brazil, the RGLP infestations maintain in high levels in Central region, during the dry season. The parasitoid constraints have shown it is necessary work considering IPM system, integrating different control methods, mainly with parasitoids, predators or entomopathogens or new natural enemies should be sought after in Australia as this area also had dry conditions.

## Acknowledgements

To IPEF (Forest Studies and Research Institute); Brazilian Forest Companies, “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)” and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)” for financial support.

## References

Berti Filho, E., V. S. Costa, R. L. Zuparko, and J. LaSalle. 2003. Ocorrência de *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) no Brasil. Revista de Agricultura. 78: 304.

Daane, K. M., K. R. Sime, D. L. Dahlsten, J. W. Andrews, and R. Zuparko. 2005 The biology of *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae), a parasitoid of the red gum lerp psyllid (Hemiptera: Psylloidea). Biol. Contr**.** 32: 228-235.

Dahlsten, D. L., S. H. Dreistadt, R. W. Garrison, and R. J. Gill. 2003. Pest notes: Eucalyptus redgum lerp psyllid. Oakland, Univ. Calif. Agric. Nat. Res. Publ. 7460. 4 p.

Dias, T. K. R., C. F. Wilcken, E. P. Soliman, H. R. Gil-Santana, and B. Zache. 2012. Occurrence of *Atopozelus opsimus* preying on nymphs and adults of *Glycaspis brimblecombei*. Phytoparasitica. 40: 137–141.

Erbilgin, N., D. L. Dahlsten, and P. Chen. 2004. Intraguild interactions between generalist predators and an introduced parasitoid of *Glycaspis brimblecombei* (Homoptera: Psylloidea). Biol. Contr. 31: 329–337.

Ferreira Filho, P. J. Dinâmica populacional do psilídeo-de-concha *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae) e parasitismo por *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) em floresta de eucalipto. 2010. Thesis (Doctorate in Plant Protection) – FCA/UNESP.

Firmino-Winckler, D. C., C. F. Wilcken, C. A. Matos, and N. C. Oliveira. 2009. Biologia do psilídeo-de-concha *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae) em *Eucalyptus* spp. Revista Brasileira de Entomologia**.** 53: 144–146.

Plascencia-Gonzalez, A., D. Cibrian-Tovar, C. Llanderal-Cazares, V. Lopez-Perez and V. Arriola-Padilla. 2005. Biologia del parasitoide *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae). Revista Chapingo. Serie Ciencias Forestales y del Ambiente. 11: 11–17.

Pogetto, M. H. F. A. D., C. F. Wilcken, M. J. Gimenes, R. S. Christovam, and E. P. Prado. 2011. Control of red-gum lerp psyllid with formulated mycoinsecticides under semi-field conditions. Int. J. Trop. Insect Sci. 31: 85–91.

Sime, K. R., K. M. Daane, D. L. Dahlsten, J. W. Andrews, and D. L. Rowney. 2004. Constraints on the effectiveness of *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae), a biological control agent for the red-gum lerp psyllid (Hemiptera: Psylloidea) in California, pp. 141–144. *In* M.S. Hoddle (ed.), Proceedings of the Fourth California Conference on Biological Control, Berkeley, USA.

Wilcken, C. F., E. B. Couto, C. Orlato, P. J. Ferreira Filho, and D.C. Firmino. 2003. Ocorrência do psilídeo-de-concha (*Glycaspis brimblecombei*) em florestas de eucalipto no Brasil. Circular técnica Ipef, (201): 1–11. (http://www.ipef.br/publicacoes/ctecnica - 23/01/2004).

Wilcken, C. F., L. A. N. Sá, M. H. F. A. D. Pogetto, E. B. Couto, P. J. Ferreira-Filho, and D.C. Firmino-Winckler. 2010. Sistema de criação do psilídeo-de-concha *Glycaspis brimblecombei* e de seu parasitóide *Psyllaephagus bliteus*, Documentos técnicos IPEF, 2: 1–23.

# Classical biological control of hemlock woolly adelgid: A complex and challenging effort in progress

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## The Pest and Initial Efforts in Biological Control

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae) burst on the scenes in the mid-1980's as a major tree-killing pests of *Tsuga* spp. in the eastern USA (Salom et al. 1996). It is native to Asia and western North America, and through DNA analysis, was determined to have been accidentally introduced into the eastern USA from southern Japan (Havill et al. 2006). Research and implementation of classical biological control has been a priority for the past two decades (Onken and Reardon 2011). The family Adelgidae lacks parasitoids (Clausen 1978) and the search for an effective HWA biological control agent has focused primarily on specialized insect predators in the Coccinellidae (*Sasajiscymnus* and *Scymnus* spp.), Derodontidae (*Laricobius* spp.), and Chamaemyiidae (*Leucopis* spp.) in Asia (Japan, China, and Taiwan) and western North America.

Primarily, two species have been released for experimental and operational purposes in the eastern USA - *Sasajiscymnus tsugae* (Sasaji and McClure) and *Laricobius nigrinus* Fender. According to the HWA Predator Release and Monitoring Database (2012), 2,413,159 *S. tsugae* have been released at 731 sites since 1998 and 164,381 *L. nigrinus* have been released at 345 sites since 2003. Two other species, *Scymnus sinuanodulus* Yu and Yao and *Scymnus ningshanensis* Yu and Yao (both from China) have been released to a much lesser extent (Montgomery and Keena 2011). *Sasajiscymnus tsugae* and *L. nigrinus* have established to varying degrees as localized populations throughout a considerable portion of the current range of HWA. Establishment has been inconsistent for *S. tsugae* (McClure and Cheah 2003; Hakeem et al. 2010); while more consistent for *L. nigrinus*, especially in plant hardiness zones 6a and 6b (Mausel et al. 2010; Davis et al. In press). At this time, evidence of suppression is limited to anecdotal observations (Cheah et al. 2005; McDonald et al. 2008). Efforts are underway to evaluate impact in a rigorous scientific fashion (Mech et al. 2010; Davis 2011), yet long-term studies are required over a variety of locations due to numerous uncontrollable variables that affect the interaction of the predators, their prey, the host trees, and the sites they grow in.

## Unintended Consequence

An unintended consequence of the widespread release of *L. nigrinus* has been its interaction with the native *Laricobius rubidus* LeConte, a predator of pine bark adelgid, *Pineus strobi* Hartig (Hemiptera: Adelgidae) on white pine, *Pinus strobus* L. *Laricobius rubidus* is commonly found feeding on HWA (Montgomery and Lyon 1996), and can complete its development on this host (Zilahi-Blogh et al. 2005). The closely related congeners can mate with each other, resulting in hybrids that can reproduce (Havill et al. In press). Currently we are investigating the introgression of hybrids in both hemlock and white pine stands in an effort to predict the long-term consequences of hybridization between a native and introduced predator.

## Additional Agents

Additional biological control agents are also being investigated. Most of the emphasis has been on the recently discovered *Laricobius osakensis*, Montgomery and Shiyake, (Montgomery et al. 2011). *Laricobius osakensis* is considered a principal predator of HWA in southern Japan (Lamb et al. 2011). Host-range tests have shown it to be specific to Adelgidae and only able to complete development on HWA (Vieira et al 2011). Also, *L. osakensis* larvae demonstrated a higher functional response and females a higher numerical response when compared to *L. nigrinus* (Vieria et al. 2012). There is no evidence that *L. osakensis* can successfully hybridize with *L. nigrinus* or *L. rubidus* (M. Fischer, unpublished studies). Permission to remove this predator from quarantine was granted in 2010. Fall 2012 is targeted for the first open releases of *L. osakensis*. The insects will be released in field settings designed to serve as field insectaries.

Another insect currently being studied in quarantine is *Scymnus coniferarum* Crotch (Coleoptera: Coccinellidae), a predator of Adelgidae native to western North America, and found on both pine (Gordon 1976) and hemlock species (Montgomery et al. 2009). Larvae feed on the progrediens lifestage and sistens eggs. This would complement the *Laricobius* spp. that feed on developing sistens and progrediens eggs. Based on an effort to rear the insect in the lab, it was possible to verify that *S. coniferarum* is able to complete development on HWA. Based on these initial findings, a petition for interstate movement has been submitted to USDA, APHIS. At the time of this writing, the petition is still pending. Ongoing assessment of host-range, biology, and rearing of this predator are necessary if this is to be considered for future biological control releases.

## Integrating Biological and Chemical Controls: Sustainable Efficacy

Long-term impact studies of the predator are ongoing, but it is apparent that at many of the release locations where *L. nigrinus* have established, older, mature trees have succumbed to HWA. The younger, more vigorous understory trees do not decline as quickly, and appear to sustain growing populations of *L. nigrinus*.

Imidacloprid has been the standard insecticide for application against HWA in urban and other settings where individual trees are highly valued. Merit 75WP and more recently Advance Tree and Shrub (Bayer®) for homeowners have been used effectively in soil applications. Recent formulation advances by Bayer have included CoreTect®, slow-release tablets placed under the organic layer around the root collar of trees. This recently registered product allows for a much easier application of imidacloprid and makes treatment of trees in remote areas more feasible. There have been some very large chemical application programs for control of the hemlock woolly adelgid. Of special note is activity in the Great Smoky Mountains National Park, where over 132,000 trees were treated once between 2002-2009 and 20,000 trees were re-treated in 2010 (Webster 2010). While providing short-term protection, this approach alone is not sustainable nor practical at the ecosystem level.

Since HWA is present on treated trees only when imidacloprid residues are extremely low, it is felt that predators will not be directly impacted in areas where trees are being protected with systemically applied chemical treatments. This lead us to consider integrating chemical and biological controls. The approach we propose is to maintain the health of a select number of large hemlocks with insecticide applications, and at the same time, release and allow the biological control agents to become established on understory trees, increase in numbers, and serve as long-term suppressers of HWA. We hypothesize that this integrated approach could save more hemlock trees over time in a given area than the use of either control treatment (biological and chemical) in isolation. If shown to be an improvement over current strategies, it can become the standard approach to area-wide IPM for HWA. We are currently testing this hypothesis at three separate locations in Kentucky, West Virginia, and Tennessee (Salom et al. 2011). If we can show that suvivorship of older and younger age classes of trees improves over time, then we will begin to recommend to foresters and other land managers to consider implementing this IPM approach.

## References

Cheah, C. A. S.- J., M. A. Mayer, D. Palmer, T. Schudder, and R. Chianese. 2005. Assessments of biological control of hemlock woolly adelgid with *Sasajiscymnus (Pseudoscymnus) tsugae* in Connecticut and New Jersey. pp. 116-130. *In* Onken, B. and R. Reardon (Compilers). Proceedings of the Third Symposium on Hemlock Woolly Adelgid in the Eastern United States. USDA For. Serv. FHTET-2005-01.

Clausen, C. P. 1978. Chermidae. Introduced Parasites and Predators of Arthropod Pests and Weeds: A World Review. USDA Agriculture Handbook. No. 480. p. 49–55.

Davis, G. A. 2011 Post-release evaluation of *Laricobius nigrinus* for the biological control of hemlock woolly adelgid in the eastern United States. Ph.D. dissertation. Virginia Tech, Blacksburg, VA. 145 pp.

Davis, G. A., S. M. Salom, C. C. Brewster, B. P. Onken, and L. T. Kok. Post-release spatiotemporal distributions of *Laricobius nigrinus* (Coleoptera: Derodontidae), a biological control agent released for hemlock woolly adelgid. Agric. For. Entomol. (In press).

Gordon, R. D. 1976. The Scymnini (Coleoptera Coccinellidae) of the United States and Canada: key to genera and revision of *Scymnus, Nephus and Diomus*. Bull. Buffalo Soc. Nat. Sci. 28: 1–362.

Havill, N. P., M. E. Montgomery, G. Yu, S. Shiyake, and A. Caccone. 2006. Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. Ann. Entomol. Soc. Am. 99: 195–203.

Havill, N. P., G. Davis, D. Mausel, J. Klein, R. McDonald, C. Jones, M. Fischer, S.t Salom, and A. Caccone. Hybridization between a native and introduced predator of Adelgidae: An unintended consequence of classical biological control. Bio. Control. (In press).

HWA Predator Release and Monitoring Database. 2012. Virginia Tech and USDA Forest Service. 24 Feb. 2012. http://hwa.ento.vt.edu/hwa/hwa.cgi

Lamb, A., M. E. Montgomery, L. C. Vieira , S. Shiyake, and S. Salom. 2011. *Laricobius osakensis,* a hemlock woolly adelgid predator from Japan. pp. 90–96. *In* Onken, B. and R. Reardon (eds.) Implementation and Status of Biological Control of the Hemlock Woolly Adelgid. FHTET 2011- 04.

McDonald, R., D. Mausel, S. Salom, L. Kok, M. Montgomery, G. Luker, S. Steury, G. Spears, S. Skeate, James Graham, and B. Hamstead. 2008. Recovery of hemlock woolly adelgid predators in the high country of northweastern North Carolina, 2004-2008. pp. 53-60. *In* Onken, B. and R. Reardon (Compilers). Proceedings of the Fourth Symposium on Hemlock Woolly Adelgid in the Eastern United States. USDA For. Serv. FHTET-2008-01.

Mausel, D. L., S. M. Salom, L. T. Kok, and G. A. Davis. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the eastern United States. *Environ. Entomol.* 39: 440–448.

Mech A. M., C. E. Jones, J. R. Rhea, J. L. Hanula, R. O. Teskey, and K. J. K. Gandhi. 2010. Status of hemlock woolly adelgid biological control beetles in the southernmost range of hemlocks. p. 172. *In* Onken, B. and R. Reardon (Compilers). Proceedings of the Fifth Symposium on Hemlock Woolly Adelgid in the Eastern United States. USDA For. Serv. FHTET-2010-07.

Montgomery, M. E., and S. M. Lyon. 1996. Natural enemies of adelgids in North America: their prospect for biological control of *Adelges tsugae* (Homoptera: Adelgidae). pp. 89-102. *In* Salom, S. M. ,T. C. Tigner, and R. C. Reardon. (eds.). Proceedings of the First Hemlock Woolly Adelgid Review. Charlottesville, VA. For. Serv. FHTET 96-10.

Montgomery, M. E., and M. A. Keena. 2011. *Scymnus* (*Neopullus*) lady beetles from China. pp. 53-76. *In* Onken, B. and R. Reardon (eds.) Implementation and Status of Biological Control of the Hemlock Woolly Adelgid. FHTET 2011- 04.

Montgomery, M. E., R. McDonald, and L. Schwartzberg. 2009. *Scymnus (Pullus) coniferarum* (Coleoptera: Coccinellidae): an adelgid predator native to the western United States. p 89. *In* Proceedings. 20th U.S. Department of Agriculture Interagency Research Forum on Invasive Species. Annapolis, MD. General Technical Report NRS-P-51.

Onken, B. And R. Reardon (eds.). 2011. Implementation and Status of Biological Control of the Hemlock Woolly Adelgid. USDA Forest Service, FHTET-2011-04.

Salom, S. M. ,T. C. Tigner, and R. C. Reardon. (eds.). 1996. Proceedings of the First Hemlock Woolly Adelgid Review. Charlottesville, VA. USDA Forest Service. FHTET 96-10.

Salom, S., A. Mayfield, and T. McAvoy. 2011. Integrating chemical and biological control. pp. 199-201. *In* Onken, B. and R. Reardon (Tech. Coord.) Implementation and Status of Biological Control of the Hemlock Woolly Adelgid. USDA Forest Service FHTET 2011-04.

Vieira, L. C., T. J. McAvoy, J. Chantos, A. Lamb, S. M. Salom, and L. T. Kok. 2011. Host range of *Laricobius osakensis* (Coleoptera: Derodontidae), a new biological control agent of hemlock woolly adelgid (Hemiptera: Adelgidae). *Environ. Entomol.* 40: 324–332.

Vieira, L. C., S. M. Salom, and L. T. Kok. 2012. Functional and numerical response of *Laricobius* spp. predators (Coleoptera: Derodontidae) on hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae). *Bio. Control.* 61: 47–54.

Webser, J. 2010. Management of the hemlock woolly adelgid in Great Smoky Mountains National Park. pp. 57-60. *In* Onken, B. and R. Reardon (Compilers). Proceedings of the Fifth Symposium on Hemlock Woolly Adelgid in the Eastern United States. USDA For. Serv. FHTET-2010-07.

Zilahi-Balogh, G. M., C. D. Broeckling, L. T. Kok, and S. M. Salom. 2005. Comparison between a native and exotic adelgid as hosts for *Laricobius rubidus* (Coleoptera: Derodontidae). *Biocon. Sci. Technol.* 15: 165–171.

# The ecology and behaviour of the parasitoid *Ibalia leucospoides*: lessons for biological control of forest pests

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What attributes of parasitoid biology are especially relevant to host regulation and limitation in classical biological control, are a matter of much research and yet little consensus. For invasive forest pests, classical biological control is a vital tool in management practices largely because of the broad time and spatial scales usually involved. The parasitoid wasp *Ibalia leucospoides* (Hochenwarth) (Hymenoptera: Ibalidae) is well known because of its role as one of several biological control agents of *Sirex noctilio* Fabricius (Hymenoptera: Siricidae). The woodwasp *S. noctilio* is probably the most damaging insect pest of cultivated pines throughout the Southern Hemisphere (Villacide & Corley 2012). While biological control success of this forest insect has been attributed largely to the effects of the introduced parasitic nematode *Deladenus siricidicola* Bedding (Tylenchida: Neotylenchidae) on wasp reproduction, the contribution of parasitoid species to woodwasp population suppression is less clear. *Ibalia leucospoides* is a solitary parasitoid that is present in all pine plantations established in Patagonia, having spread together with *Sirex noctilio*. Parasitism levels have been estimated to reach approximately 40% locally, but it has been observed that these levels vary strongly both spatially and temporally. Also, pest population outbreaks have been experienced, even when the parasitoid was present.

Our aim is, in continuation of recent work (Corley 2011), to present those ecological features of this parasitoid that may be involved in its success as a natural enemy of *S. noctilio*. By looking first into the pest's ecology, we then focus on three major aspects: host searching (Fischbein et al 2012a; Pietrantuonno et al 2012), dispersal and life history (Corley & Bruzzone 2009; Fischbein et al 2012b). Together, we expected through the analysis of recent data on these aspects, to contribute to more accurately establish our expectations on the role played by this parasitoid in regulating woodwasp populations affecting pine forestation worldwide.

## References

Corley J. C. 2011. On cues, food, competition and development. An overview of the behavior and life history traits of *Ibalia leucospoides* and their implications for the bio-control of *Sirex noctilio*. *IUFRO* Forest Protection Joint Meeting, Colonia, Uruguay.

Corley J. C., and O. A. Bruzzone. 2009. Delayed emergence and the success of parasitoids in biological control. Biol. Control 51: 471–474.

Fischbein, D., C. Bernstein, and J. C. Corley. 2012. Linking reproductive and feeding strategy in the parasitoid *Ibalia leucospoides*: Does feeding always imply profit? Evol. Ecol. (online; DOI 10.1007/s10682-012-9608-9).

Fischbein, D., J. Bettinelli, C. Bernstein, and J. C. Corley. 2012. Patch choice from a distance and use of habitat information during foraging by the parasitoid *Ibalia leucospoides* (Hymenoptera, Ibaliidae). Ecol. Entomol. 37:161-168.

Pietrantuono, A, L., V. Fernández-Arhex, N. Jofré, and J. C. Corley. 2012. Food and host searching by *Ibalia leucospoides* (Hymenoptera: Ibaliidae), a parasitoid of *Sirex noctlio* (Hymenoptera: Siricidae). J. Insect Behav. 25: 320–327.

Villacide, J. M., and J. C. Corley. 2012. Ecological knowledge of the woodwasp *Sirex noctilio*: tackling the challenge of successful pest management. Int. J. Pest Manage. 58: 249–256.

# Session 9: The Effect of Climate Change on Biological Control

## Predicting the effects of climate change on mealybugs and their natural enemies in grapevines in Australia

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Understanding the consequences of climate change on pest insects and their natural enemies will be essential for biological control in the near future. The effectiveness of natural enemies in controlling pests will decrease if pest distributions shift into enemy free space outside the distribution of their natural enemies, although a new community of enemies might then provide some level of control (Thomson et al. 2010). Pest damage will depend on individual pest effects, changes in pest complexes and relevant natural enemies.

Mealybugs are important vineyard pests, and increasing in both their distribution extent and abundance. Mealybugs are an economic problem in grape vines because of direct damage to the crop and costs for their control, but also their role in transmitting grapevine leafroll viruses. Three mealybugs commonly occur in Australian vineyards including two native species, longtailed mealybug, *Pseudococcus longispinus* (Targioni Tozzetti), and citrophilus mealybug, *Pseudococcus calceolariae* (Maskell) (Hemiptera: Pseudococcidae). The third species in Australia, *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae), is obscure, however recent analysis based on parasitoids is consistent with a South American origin(Charles 2011), although *P. viburni* is known to have been in Australia since as early as 1893 (Maskell 1894). Though records in Australia for the three species are patchy, longtailed mealybug appears to be the most widely distributed, recorded from all states of Australia and from a variety of host plants. There are also looming biosecurity risks such as vine mealybug *Planococcus ficus* (Signoret) and grape mealybug *Pseudococcus maritimus* (Ehrhorn) (Hemiptera: Pseudococcidae). Vine mealybug has shown itself to be an efficient invader, now a key pest in Europe, Africa, South Africa, Argentina, the Middle East, California and Mexico, and currently regarded as a potential threat to the Australian grape industry. Vine mealybug has a number of traits that may make it particularly damaging and difficult to control, especially under climate change scenarios, including a potential for faster population growth resulting from more generations per year. It may also be a more efficient transmitter of grapevine leafroll viruses, a cause of increasing concern in grape-growing regions globally.

References to the relationship of mealybug outbreaks to chemical use reinforces the importance of natural enemies. Mealybugs have many natural enemies including a range of parasitoids and coccinellid beetles. Generalist natural enemies may be particularly important: a recent review of biological control agent performance concluded that biological control efficacy tended to be higher when agents were generalists than when they were specialists (Stiling and Cornelissen, 2005). Parasitoids may not only be more specialized but may also attack a limited number of different life stages. For example, the native Australian longtailed mealybug parasitoid *Anagyrus fusciventris* (Girault) (Hymenoptera: Encyrtidae) parasitizes only adults (Furness 1976). A generalist predator, the ladybird beetle *Cryptolaemus montrouzieri* Mulsant ('mealybug destroyer') (Coleoptera: Coccinellidae) is a well known predator of mealybugs, and is both reared in insectaries for release during mealybug outbreaks and is introduced into other countries, e.g. in North America and South Africa, as a predator of mealybugs, including vine mealybug (Walton and Pringle 2005, Gutierrez et al. 2008).

We mapped the current distribution for longtailed mealybug and *C. montrouzieri* in Australia and built ecological niche models for each species using the correlative modelling program, MAXENT. Predictor variables useful for describing the climate space of each species were determined and the models were projected into a range of future climate change scenarios to assess how suitable climate space may shift. We also mapped the potential distribution of vine mealybug in Australia using published data (Gutierrez et al. 2008), predicting changes in *C. montrouzieri* and the vine mealybug in California. We then investigated potential distribution-limiting traits in lethal thermal limits. Individuals of both longtailed mealybug and *C. montrouzieri* were subjected to temperature treatments for 2 h using an immersion water bath to determine lethal thermal limits and these data were used to determine distributional limits across the Australian landscape. Responses of the pest and a natural enemy were compared to investigate potential changes in the control efficacy of *C. montrouzieri* on both its native prey, longtailed mealybug, and the potentially invasive vine mealybug through changes in their distribution and abundance.

## References

Gutierrez, A. P., K. M. Daane, L. Ponti, V. M. Walton, and C. K. Ellis. 2008. Prospective evaluation of the biological control of vine mealybug: refuge effects and climate. J. Appl. Ecol. 45: 524–536.

Maskell, W. M. 1894. Further Coccid notes: with descriptions of several new species, and discussion of various points of interest. T. P. Roy. Soc. New Zeal. 26: 65–105.

Stiling, P. T., and T. Cornelissen. 2005. What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. Biol. Control 34: 236–246.

Thomson, L.J., S. Macfadyen, and A. A. Hoffmann. 2010. Predicting the effects of climate change on natural enemies of agricultural pests. Biol. Control 52: 296–306.

Walton, V. M., and K. L. Pringle. 2005. Vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a key pest in South African vineyards. S. Afr. J. Enol. Vitic. 25: 54–62.

# Performance of a biological control community under extreme temperatures

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Current models of climate change predict that there will be increases in the frequency and severity of heat-waves in the coming century. These perturbations are likely to affect the composition and performance of natural enemy communities, and consequently to also affect the biological control of pests through effects on the underlying trophic cascades. These effects are likely to emerge from differential effects of extreme temperatures on individual members of these communities.

Arthropod pests and their arthropod biological control agents are poikilotherms. Body temperatures and enzymatic processes are therefore largely dependent on environmental temperature. As a consequence, environmental temperature largely drives population dynamics (Trudgill et al. 2005, Clarke 2003). Differential effects of temperature on members of a community could therefore affect many aspects of the relationships between organisms on a biological control food web. Differential effects on development rate could result in loss of synchronization between hosts and parasitoids (Davis et al. 1998). Differential effects of extreme temperatures on mortality could affect relative abundance of prey and predators. Effects of extreme high temperatures on plants, which are the basal elements of biological control food webs, may influence biological control processes as bottom-up effects. Many poikilotherms exhibit behaviors that regulate internal temperature, particularly to offset the effects of temperatures at the limits of physiological tolerance. With respect to extreme high temperatures seeking shade and immobility are common behaviours (e.g. Schultz 1998), and an increase in the frequency of such behaviours by predators could provide prey with refuges from predation.

We examined these ideas experimentally, using an aphid-based community consisting of pepper plants, *Capsicum anuum* L. (Solanaceae), green peach aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and two parasitoids: *Aphelinus abdominalis* (Dahlman) (Hymenoptera: Aphelinidae) and *Aphidius matricariae* (Haliday) (Hymenoptera: Braconidae). In general, microcosm communities were exposed to either daily or twice-weekly perturbations in temperature, with mid-day peaks at either 32°C or 40°C. Effects emerged from those studies which likely involved complex interactions between host behaviour, host performance, parasitoid behaviour and parasitoid performance under the different temperature regimes.

In studies examining the effects of temperature perturbations on components of the complex tri-trophic community (Gillespie et al. 2012) the osmolarity of pepper plant sap increased under 40°C, suggesting the emergence of compounds defending against heat shock. Aphid populations increased more slowly and the development time of parasitoids was longer, when exposed to daily extremes of 32°C and 40°C, as opposed to periodic exposure to the same extremes. When all four species in the food web were simultaneously present, the aggregate impacts of parasitoids on the aphid population were greatest when the food web was exposed to daily extremes of 32°C or 40°C, compared to periodic exposure to the same extremes. Overall, numbers of *A. matricariae* were lower when exposed to the daily extreme treatments compared to the periodic exposure to the same extremes. Numbers of *A. abdominalis* were not affected by temperature treatment directly but were affected indirectly through competition with *A. matricariae*, which was negatively affected by temperature treatment.

The underlying mechanisms affecting the performance of biological control agents may be much more complex than differential effects of temperature on development time. The costs of defense (walking, dropping) in aphids is higher when exposed to daily extremes of either 32°C or 40°C, compared to periodic exposure to the same extremes, but the foraging ability of *A. matricariae* was not affected by temperature treatments (Bannerman et al. 2011). In separate experiments, when exposed to daily extremes of 40°C, *A. abdominalis* formed mummies on the soil of enclosures, whereas mummies were formed on plants in all other conditions (A. N. and D. G., unpublished data). It is not clear if this effect is due to an influence of the parasitoid larva on the behaviour of the aphid host, or is a consequence of behaviour of the aphid.  In either case, position of mummies of *A. abdominalis* is affected by temperature and this will quite likely affect their exposure to risk of predation or hyperparasitism. To evaluate the direct effects of mortality, mummies of *A. matricarae* were exposed to short periods of exposure (5–120 min) to temperatures from 36 to 44°C. Significant mortality in mummies of *A. matricariae* was only observed at 42°C and 44°C and mummies could tolerate temperatures up to 40°C for 2 h without measurable mortality (C. H. and D. G. unpublished data). At 42°C and 44°C, surviving wasps required up to an additional day to complete development compared to individuals exposed to lower temperatures. It appears that *A. matricariae* can withstand temperatures at least as high as the reported upper lethal temperature for *M. persicae*.

Thus far, our work suggests complex and interacting effects of temperature on biological control communities. Temperature affects the physiology and behaviour of all members of these communities. A more broadly-based theory of the effects of temperature perturbation on biological control communities is clearly necessary to understand and predict the results of biological control under more variable climates in the 21st Century.  In a typically stochastic world, temperature variation has many dimensions, and these need to be considered in modelling and prediction. In Nicholson-Bailey models of an aphid-parasitoid system, the magnitude of extremes, the frequency of occurrence and the autocorrelation of high temperature events all affect the persistence of communities (J. B. and B. R., unpublished data).

## References

Bannerman, J. A., D. R. Gillespie, and B. D. Roitberg, 2011. The impacts of extreme and fluctuating temperatures on trait-mediated indirect aphid-parasitoid interactions. Ecol. Entomol. 36: 490–498.

Davis, A. J., J. H. Lawton, B. Shorrocks, and L. S. Jenkinson. 1998. Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. J. Anim. Ecol. 67: 600–612.

Clarke, A. 2003. Costs and consequences of evolutionary temperature adaptation. Trends in Ecology and Evolution 18: 573–581.

Gillespie, D. R., A. Nasreen, C. E. Moffat, P. Clarke, and B. D. Roitberg. 2012. Effects of simulated heat waves on an experimental community of pepper plants, green peach aphids and two parasitoid species. Oikos 121: 149–159.

Schultz, T.D. 1998. The utilization of patchy thermal microhabitats by the ectothermic insect predator, *Cicindela sexguttata*. Ecol. Entomol. 23: 444-450.

Trudgill, D. L., A. Honek, D. Li, and N. M. Van Straalen. 2005. Thermal time - Concepts and utility. Ann. Appl. Biol. 146: 1–14.

# Environmental conditions affect competitive and predatory interactions among natural enemies. Implications for pest biological control in a changing climate

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Climate change predicts increases in the global mean surface temperature as well as local/regional increases in the intensity, frequency, and duration of events with extreme high temperatures (IPCC Houghton et al. 2001, Parry et al. 2007). In crops where biological control is the basis of pest control (e.g. organic farming, IPM), environmental warming may provide pests with increasing chances to escape predator control (Montserrat et al. 2012a) because sensitivity to rising temperatures increases with trophic level (Voigt et al. 2003). Added to that, species interaction strength is commonly climate-dependent (Beveridge et al. 2010, Gilman et al. 2010). Indeed, environmental warming and frequent exposure to extreme environmental conditions is affecting virtually every type of interaction between species, including competitive abilities, and predation and encounter rates (Tylianakis et al. 2008, and references therein), as well as foraging behaviour and dispersal rates (Skirvin and Fenlon 2003). In fact, recent theoretical works have predicted deep changes in the dynamics and the structure of communities, as properties emerging from changes in environmental conditions (Gilman et al. 2010, Wilmers et al. 2007, Vasseur and McCann 2005).

We investigated the effect of warming in a community present in avocado, *Persea americana* Mill. (Lauraceae), orchards of south-eastern Spain. This community differs depending on the location of the avocado orchards. In coastal areas, with relatively mild environmental conditions, the community is mainly composed of the herbivore *Olygonichus perseae* (Tuttle, Baker & Abbatiello) (Acari: Tetranychidae), an invasive pest species detected for the first time in Spain in 2004, and two species of phytoseiid mites that naturally occur in Mediterranean ever-green agro-ecosystems such as citrus and avocado: *Neoseiulus californicus* (McGregor) and *Euseius stipulatus* (Athias-Henriot) (Acari: Phytoseiidae) (Wong et al. submitted, Aguilar-Fenollosa et al. 2011). In interior valley bottoms, where environmental conditions are more extreme, the most abundant phytoseiid mite co-occurring with the herbivore pest is *Euseius scutalis* (Athias-Henriot) (Acari: Phytoseiidae) (Wong et al., submitted), a species known to be highly tolerant to hot and dry environmental conditions (Kasap & Şekeroğlu 2004). In this work, we examined 1) whether variation in predator-predator interaction strength (i.e. competition and/or predation) caused by environmental conditions could explain the observed distribution and abundance of the sister species *E. stipulatus* and *E. scutalis*; and 2) whether climate-dependent species interaction strength could explain the disruption of biological control in coastal avocado orchards.

1) We carried out laboratory population dynamics experiments, and experiments at an individual level, to determine which of the two *Euseius* spp. was superior at exploitative competition for a resource that both can exploit, and to assess how resource exploitation efficiency varied depending on environmental conditions. Results suggested that when the two species co-occur under hot and dry climate scenarios, *E. scutalis* should always exclude *E. stipulatus*. Community dynamic

experiments with the two species together at hot and dry environmental conditions confirmed this hypothesis, which is in agreement with the pattern observed in the field. At milder environmental conditions, the population of the two species reached similar levels, suggesting that under these conditions both species may exploit the resource with similar efficiency. However, in the community dynamics experiments with the two species together, the tendency was always towards *E. scutalis* being excluded by *E. stipulatus*, a result that is also in agreement with what is observed in the field, but that cannot be explained by differences in competitive abilities between the species. Yet, it is a result that can be observed when competing predators are involved in predator-prey interactions (Montserrat et al. 2012b, 2008). We concluded that while predator-prey interactions between the two species (i.e. intraguild predation, reciprocal intraguild predation – Polis et al. 1989) are crucial factors determining the structure of the community when environmental conditions are mild, tolerance to thermal stress, which may confer competitive and/or predatory superiority, becomes essential when environmental conditions are severe. Additional lab experiments, where (reciprocal) intraguild predation rates were evaluated at different environmental conditions, are in agreement with our conclusion. Overall, our results show that climate can be key as a driver shaping the structure of communities.

2) In a coastal avocado orchard, we applied a biological control strategy aimed at enhancing the performance in the field of *E. stipulatus* and *N. californicus* as natural enemies of *O. perseae* during two consecutive years. Unexpected poor results led us to analyse additional factors involved in the dynamics of the community, such as summer environmental conditions. Non-linear regression models considering biotic (predation, intra-specific competition) and abiotic (temperature, relative humidity) effects revealed that high temperatures during the second half of the summer were central to predator/prey population decimation. Therefore, we showed that harsh environmental conditions were a direct cause of biological control disruption. Lab experiments confirmed that predatory interactions among the components of the community were strongly affected by environmental conditions, profoundly weakening the performance of both predators as natural enemies of the pest.

We believe that in the future the success of biological control strategies will be limited by the natural enemy's capacity to respond adaptively to rapid climate changes, and that research aimed at evaluating the evolutionary potential of natural enemies to rapid climate change should be the focus of near-future investigation.

## References

Aguilar-Fenollosa, E., M. V. Ibáñez-Gual, S. Pascual-Ruiz, M. Hurtado, and J. A. Jacas. 2011. Effect of Ground-Cover Management on Spider Mites and their Phytoseiid Natural Enemies in Clementine Mandarin Orchards (II): Phytoseiid Mite Dynamics. Biol. Control 59: 171–179.

Beveridge, O. S., S. Humphries and O. L. Petchey. 2010. The interacting effects of temperature and food chain length on trophic abundance and ecosystem function. J. Anim. Ecol. 79: 693–700.

Gilman, S. E., M. C. Urban, J. Tewksbury, G. W. Gilchrist, and R. D. Holt, 2010. A framework for community interactions under climate change. T. Ecol. Evol. 25: 325–331.

Houghton, J. T., Y. Ding, D. J. Griggs, M. Noguer, P. J. van der Linden, X. Dai, K. Maskell and C. A. Johnson. 2001. Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Parry, K. L., O. F. Canziani, J. P. Palutikif, P. J. van der Linden, and C. E. Hanson (eds.). 2007. Climate change 2007: Impacts, adaptation and vulnerability.

Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, , Cambridge University Press, Cambridge, UK.

Kasap, I., and E. Sekeroglu, 2004. Life history of *Euseius scutalis* feeding on citrus red mite *Panonychus citri* at various temperatures. BioControl 49: 645–654.

Montserrat, M., R. M. Sahún, and C. Guzmán. 2012. Can climate change jeopardize predator control of herbivore invasive species? A case study in avocado agro-ecosystems in Spain. Exp. Appl. Acarol. (in press) DOI: 10.1007/s10493-012-9560-y.

Montserrat M., S. Magalhâes, M. W. Sabelis, A. M. de Roos, and A. Janssen. 2012. Invasion success in communities with reciprocal intraguild predation depends on the stage structure of the resident population. Oikos 121: 67–76.

Montserrat M., S. Magalhâes, M. W. Sabelis, A. M. de Roos, and A. Janssen. 2008. Patterns of exclusion in an intraguild predator-prey system depend on initial conditions. J. Anim. Ecol. 77: 624–630

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Ann. Rev. Ecol. Evol. Syst. 20: 297–330.

Skirvin D. J., and J. S. Fenlon. 2003. Of mites and movement: the effects of plant connectedness and temperature on movement of *Phytoseiulus persimilis.* Biol. Control 27: 242–250.

Tylianakis J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. Ecol. Let. 11: 1351–1363.

Vasseur, D. A., and K. S. McCann. 2005. A mechanistic approach for modeling temperature-dependent consumer-resource dynamics. Amer. Nat. 166: 184–198.

Voigt, W., J. Perner, A. J. Davis, T. Eggers, J. Schumacher, R. Bahrmann, B. Fabian, W. Heinrich, G. Kohler, D. Lichter, R. Marstaller, and F. W. Sander. 2003. Trophic levels are differentially sensitive to climate. Ecology 84: 2444–2453.

Wilmers C. C., E. Post, and A. Hastings. 2007. The anatomy of predator-prey dynamics in a changing climate. J. Anim. Ecol. 76: 1037–1044.

Wong, E., J. M. Vela, M. Montserrat, and J. R. Boyero. Parapatry of two *Euseius* species in avocado groves in Southeastern Spain (submitted).

# Climate change and biological control: case studies from New Zealand

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Natural enemies are one of the primary drivers determining the abundance and fitness of a species within an ecosystem. When an exotic plant or animal becomes established in a relatively simple ecosystem without its co-evolved natural enemies, it often reaches higher populations or has more vigorous growth than in its country of origin. As a geographically-isolated island nation with a temperate maritime climate, New Zealand has exploited this ecological opportunity, resulting in a robust economy based on agricultural, forestry and horticultural export industries heavily dependent on exotic plant and animal species. Likewise, the absence of natural enemies and little competition contributes to New Zealand's susceptibility to invasive exotic weeds and invertebrates and, in turn, the success of its classical biological control programmes. New Zealand relies on biological control to export high quality produce into internationally competitive markets under tight quarantine and pesticide residue restrictions, to conserve natural habitats and to foster sustainable systems dependent on natural processes with minimal intervention.

New Zealand is a long, narrow island nation spanning a range of different climate zones, from warm subtropical in the far north to cool temperate in the far south, and severe alpine conditions in the mountainous areas. By 2090, its climate is predicted to be around 2°C warmer, on average, than in 1990. Rainfall is expected to increase in the west and decline in the east, and extreme weather events may be more common. The likely impacts of climate change projections to 2090 in differing primary production sectors were investigated.

Climate change will have a direct effect on the location, nature and productivity of the pastures, crops, orchards and forests grown in the future in New Zealand. The key climate change challenges identified were: 1) disparities in natural enemy capability to migrate; 2) milder winters leading to emergence of new pests and additional pest generations; 3) non-target impacts on native species due to range and temperature changes; 4) increased disruptions caused by extreme weather events; 5) disruption of host: natural enemy synchrony; and 6) insufficient genetic diversity to allow evolutionary adaptation.

The potential impacts of climate change were examined for five case study biological control systems.

- Ragwort, *Jacobaea vulgaris* Gaertner (Asteraceae), suppression by ragwort flea beetle *Longitarsus jacobaeae* Waterhouse (Coleoptera; Chrysomelidae) is likely to fail when mean annual rainfall exceeds 1670 mm (Gourlay et al. 2008). Climate change will increase the area in western regions where biological control will fail but larger areas in northern North Island will attain suppression. In the eastern regions, the area increases where ragwort will not grow.
- Tomato fruitworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is predicted to have a problematic extra autumn generation over much of the North Island, potentially limiting the viability of late corn and processing tomato

*Proceedings of the 4th International Symposium on Biological Control of Arthropods,* Pucón, Chile.
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crops. Given that the key parasitoid, *Cotesia kazak* (Telenga) (Hymenoptera: Braconidae), has not been effective at controlling this pest in warmer areas overseas, the future viability of the current Integrated Pest Management (IPM) systems may be compromised.

- Woolly apple aphid *Eriosoma lanigerum* (Hausmann) (Hemiptera: Pemphigidae) may achieve elevated densities in early spring before its parasitoid *Aphelinus mali* Haldeman (Hymenoptera: Aphelinidae) becomes active after diapause, but greater suppression may be possible over the summer and autumn (Shaw and Walker 1996, Shaw and Wallis 2009). The overall effectiveness of the IPM programme is predicted to be maintained, but with increasing requirement for an insecticide application in spring.
- The current successful suppression of lucerne weevil, *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae), by *Perilitus aethiops* Nees [=*Microctonus aethiopoides* Loan] (Hymenoptera: Braconidae) (Goldson, Proffitt, and McNeill, 1990) may be compromised as New Zealand's climate approaches that of South Australia, where the biological control agent currently fails to suppress the pest.
- Continuing biological control suppression of the Argentine stem weevil *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) may rely on local adaptation of the parasitoid *Perilitus hyperodae* (Loan) [=*Microctonus hyperodae* Loan] (Hymenoptera: Braconidae) biotype complex (Phillips et al. 2008). The low genetic variability of many other biological control agent populations may limit their ability to adapt successfully to future conditions.

A number of actions are recommended to ensure biological control continues as a mainstream pest management tool in New Zealand, including: refuge habitats; pre-emptive action against pests currently kept in check by low winter temperatures; introductions of new genetic lines for introduced biological control agents founded by few individuals; and continued border biosecurity and surveillance, backed by rapid response, to defend against the increased risk that the frequent subtropical "door knockers" become permanently established.

## References

Gourlay, A. H., S. V. Fowler, and G. Rattray. 2008. Is ragwort flea beetle (*Longitarsus jacobeae*) performance reduced by high rainfall on the West Coast, South Island, New Zealand?, pp. 545–551 *In* Julien. M.H., R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz, and B.G. Rector (eds.) Proceedings of the XII International Symposium on Biological Control of Weeds. CAB International, Wallingford, UK.

Goldson, S. L., J. R. Proffitt, and M. R. McNeill. 1990. Seasonal biology and ecology in New Zealand of *Microctonus aethiopoides* (Hymenoptera: Braconidae), a parasitoid of *Sitona* spp. (Coleoptera: Curculionidae), with special emphasis on atypical behaviour. J. Appl. Ecol. 27: 703–722.

Phillips, C. B., D. B. Baird, I. I. Iline, M. R. McNeill, J. R. Proffitt, S. L. Goldson, and J. M. Kean, 2008. East meets west: adaptive evolution of an insect introduced for biological control. J. Appl. Ecol. 45: 948–956.

Shaw, P. W., and J. T. S. Walker 1996. Biological control of woolly apple aphid by *Aphelinus mali* in an integrated fruit production programme in Nelson, pp. 59–63 *In* Proceedings of the Forty Ninth New Zealand Plant Protection Conference.

# Genotype matching in a parasitoid-host genotypic food web: an approach for measuring effects of environmental change

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Despite strong arguments that food-web structure can shape coevolution (Thompson 1994), food-web research to date has focused almost entirely on interactions (links) between multiple species (nodes) (Bascompte 2009). In contrast, natural selection operates by altering the frequencies of genes within populations of a single species, and whether environmental changes can alter the within-species preferences between genotypes remains unknown. The preference of certain consumer genotypes for specific prey genotypes may drive species divergence, evolutionary arms races or even speciation (Feder and Forbes 2010). Therefore, understanding the interplay between environmental forces, frequencies of genes, and food-web interactions among species, will be critical for determining the eco-evolutionary outcomes of diverse environmental forces (e.g. global environmental change). Climate imposes a major selective force on organisms, altering various aspects of their biology (Parmesan 2006) and interactions with other species (Tylianakis et al. 2008). Yet, all species are embedded in networks of feeding interactions (food webs) with other species (Bascompte 2009), which can potentially alter evolutionary responses to environmental drivers such as climate (Ives 1995, Harmon et al. 2009). Furthermore, food-web structure can determine emergent properties such as ecosystem stability (McCann 2000, de Ruiter et al. 2005, Bascompte 2009), but this structure can be altered by human-induced changes to the environment (Montoya et al. 2006, Tylianakis et al. 2007), such as climate warming (de Sassi et al. 2012).

Here we sampled host aphid colonies across a natural climatic gradient in Chile, reared hosts for the emergence of parasitoids, and genotyped interacting host and parasitoid individuals (Lavandero et al. 2009; Lavandero and Dominguez 2010; Lavandero et al. 2011). Finally, we used feeding associations between host and parasitoid genotypes to generate a ‘genotypic food web’. As a case example of the kinds of questions that can be addressed with genotypic food webs, we use a method for quantifying congruence between the genetic relatedness of parasitoid individuals and that of the hosts they attack (Legendre et al. 2002). Then we use a natural climatic gradient to show that a reduced number of frost days could increase genotypic congruence within replicated interaction networks involving different genotypes of a single specialized parasitoid species and its aphid host (Figure 1).

Here we show that a food web incorporating individuals and their genotypes can uncover changes to host-genotype use by parasitoids, with potential future consequences for evolution and functional resilience. In cases where genotypic similarity translates into phenotypic similarity (i.e. functional traits), elevated genotypic congruence within interaction networks could accelerate the loss of functional groups, as related species (or in this case, genotypes) go extinct from the network (Rezende et al. 2007). Furthermore, reduced plasticity in host use by parasitoids could reduce the resilience of food webs and biological control to changes in host-genetic structure or to invasion by novel host genotypes. In cases of stronger host and parasitoid genotype congruence (such as we observed in warmer climates), the effectiveness of biological control programmes could be altered. Furthermore, classical biological control has traditionally been carried out with poor information on environmental matching (e.g.

climatic tolerance) and with limited genetic material (small introduced populations of inbred natural enemies such as parasitoids). This low genetic variability could limit the ability of parasitoids to cope with a broad range of host genotypes.

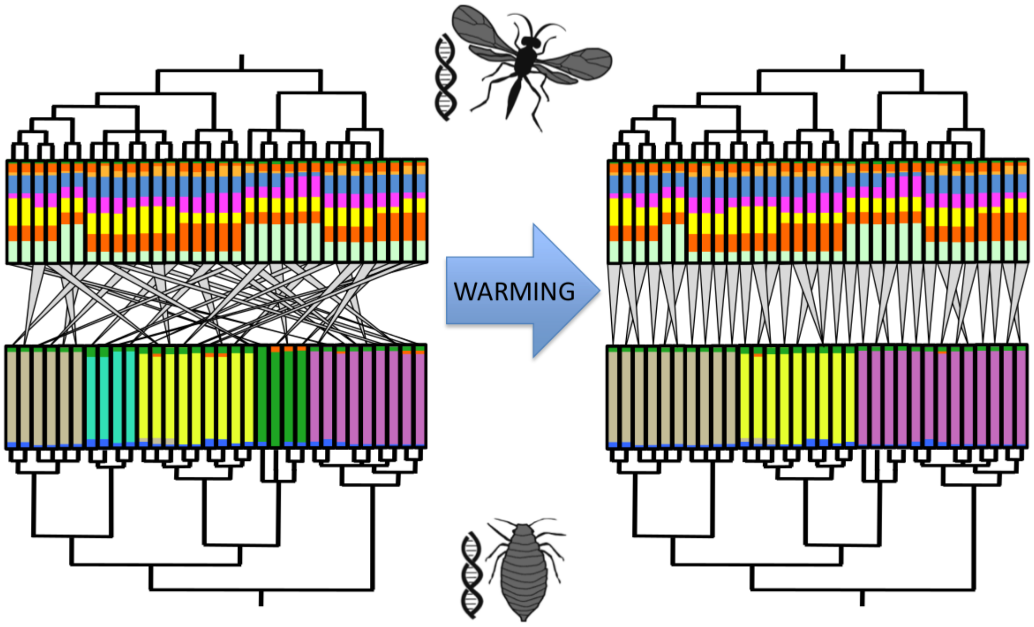

Figure 1. Illustration of the tested hypothesis that genotype congruence of a genotypic food web changes with temperature. The top row represents parasitoid genotypes and the bottom row host aphid genotypes (the parasitoid and host each comprise one species). Average assignment probability of individuals of the host and the parasitoid (independent from sampling origin) are represented by different colours (representing different genetic clusters). Distances between individuals based on shared alleles (i.e. genetic distances) are represented by the trees. Links between a parasitoid and host represent a feeding interaction. The food web on the left shows genotypically-random associations between hosts and parasitoids, characteristic of cooler sites. As temperature increases (fewer frost days), we found greater phylogenetic congruence, such that closely-related parasitoids attacked closely-related hosts (right food web) (source: Mol. Ecol. 22: 229-238).

Thus, in addition to the potential disruption of species interaction networks through altered phenology and behaviour (Memmott et al. 2007, Tylianakis et al. 2007, 2008), the environment may alter within-species selection pressures and resilience within food webs, and detection of such effects will require within-species genotype analyses. We hope that, with the growing power and declining cost of high-throughput sequencing technology (Schlotterer 2004), future studies will be able to examine genotype food webs nested within species-level food webs, or apply this technique to other kinds of species interactions such as mutualisms or competition, thereby gaining a more complete understanding of the interplay between the environment, evolutionary adaptation and ecological interactions.

## Acknowledgements

This research was supported by FONDECYT (Project Number 11080013), Capital Humano Avanzado-MEC of CONICYT, and an International Foundation for Science grant to BL. JMT is funded by the Marsden Fund of New Zealand (UOC-0705) and a Rutherford Discovery Fellowship, administered by the Royal Society of New Zealand. We would like to thank Jordi Bascompte, Christian Figueroa, Pierre Frank, Eduardo Fuentes, Claudio Ramirez, Daniel Stouffer and Christoph Vorburger for extremely useful comments on previous drafts of the manuscript, Paul Gardner and Daniel

Stouffer for discussions on the analysis, and Matt Walters and Angela Mendez for help with figures.

## Data Accessibility

Individual genotypes of aphid and corresponding emerged parasitoids per site, interaction matrices and distances matrices can be obtained trough doi:10.5061/dryad.k0d7s. Climatic data used was obtained from http://www.dgf.uchile.cl/PRECIS/#Acceso_a_los_datos. Sampling details (Table S1 and 2) and climatic data (Table S1) uploaded as online supplemental material.

## References

Bascompte J. 2009. Disentangling the Web of Life. Science 325: 416–419.

de Ruiter P.C., V. Wolters , J.C. Moore and K.O. Winemiller. 2005. Food web ecology: playing Jenga and beyond. Science 309: 68–71.

de Sassi C., P. P. J. Staniczenko, and J. M. Tylianakis. 2012. Warming and nitrogen affect size structuring and density dependence in a host-parasitoid food web. Philosphical Trans. R. Soc. London, B. 67: 3033–3041.

Feder J. L. and A. A. Forbes. 2010. Sequential speciation and the diversity of parasitic insects. Ecol. Entomol. 35: 67–76.

Harmon J. P., N. A. Moran, and A. R. Ives. 2009. Species response to environmental change: Impacts of food web interactions and evolution. Science 323: 1347–1350.

Ives A. R. 1995. Predicting the response of populations to environmental-change. Ecology 76: 926–941.

Lavandero B., and M. Dominguez. 2010. Isolation and characterization of nine microsatellite loci from *Aphelinus mali* (Hymenoptera: Aphelinidae), a parasitoid of *Eriosoma lanigerum* (Hemiptera: Aphididae). Insect Sci. 17: 549–552.

Lavandero B., C. C. Figueroa, P. Frank, and A. Mendez. 2011. Estimating gene flow between refuges and crops: A case study of the biological control of *Eriosoma lanigerum* by *Aphelinus mali* in apple orchards. Plos One, 6, 11, e26694.

Lavandero B., C. C. Figueroa, C. C. Ramirez, P. D. S. Caligari, and E. Fuentes-Contreras. 2009. Isolation and characterization of microsatellite loci from the woolly apple aphid *Eriosoma lanigerum* (Hemiptera: Aphididae: Eriosomatinae). Mol. Ecol. Res. 9: 302–304.

Legendre P., Y. Desdevises and E. Bazin. 2002. A statistical test for host-parasite Coevolution. Syst. Biol. 51: 217–234.

McCann K.S. 2000. The diversity-stability debate. Nature 405: 228–233.

Memmott J., P. G. Craze, N. M. Waser and M. V. Price. 2007. Global warming and the disruption of plant-pollinator interactions. Ecol. Lett. 10: 710–717.

Montoya J. M., S. L. Pimm and R. V. Sole. 2006. Ecological networks and their fragility. Nature 442: 259–264.

Parmesan C. 2006. Ecological and evolutionary responses to recent climate change. Ann. Rev. Ecol. Evol. Syst. 37: 637–669.

Rezende E. L., J. E. Lavabre, P. R. Guimarães, P. Jordano and J. Bascompte. 2007. Non-random coextinctions in phylogenetically structured mutualistic networks. Nature 448: 925–928.

Schlotterer C. 2004. The evolution of molecular markers - just a matter of fashion? Nat. Rev. Genet. 5: 63–69.

Thompson J. N. 1994. The Coevolutionary Process. University of Chicago Press, Chicago, IL, USA.

Tylianakis J. M., R. K. Didham, J. Bascompte and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. Ecol. Lett. 11: 1351–1363.

Tylianakis J. M., T. Tscharntke and O. T. Lewis. 2007. Habitat modification alters the structure of tropical host-parasitoid food webs. Nature 445: 202–205.

# Session 10: Use of Volatiles to Monitor or Manage Natural Enemies

## Semiochemicals and biological control: Complexity in nature and in pest management

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Semiochemicals are essential in the life histories of arthopod biological control agents. They also have the potential to work as management tools in many ways to promote biological control in pest management. Humans have much to learn from the extremely diverse types of semiochemical interactions seen in nature. Pest management strategies using pheromones and other semiochemicals need to recognize both potential advantages and disadvantages for biological control.

Monitoring for presence or abundance of pest species with their sex or aggregation pheromones often reduces pesticide treatments which are harmful to biological controls. When pheromones are used in mating disruption programs for pest Lepidoptera, an indirect effect is that reduced broad-spectrum pesticide use can allow natural enemies to better suppress other pests or potential pests (Witzgall et al. 2010). This welcome and beneficial effect, however, does not begin to reflect the huge diversity of semiochemical use by predatory and parasitoid arthropods, nor the potential for pest management based on these many ecological roles of semiochemicals.

Many if not all arthropod natural enemies use pheromones for intraspecific communication. Lacewings and asopine predatory bugs are some of the predators for which aggregation pheromones are known (Greenstone and Dickens 2005; Chauhan et al. 2004, 2007; Aldrich 1999). Semiochemicals may be used to attract, repel, detect, or capture (and redistribute) predators and parasitoids. For example, the aggregation pheromone of the predatory *Podisus maculiventris* (Hemiptera: Pentatomidae) can be used to capture overwintered adult bugs in the spring, which are released later into enclosures which function as predator nurseries in vegetable fields for control of Colorado potato beetle (*Leptinotarsa decemlineata* [Coleoptera: Chrysomelidae]) or cabbage looper (*Trichoplusia ni* [Lepidoptera: Noctuidae]) (Aldrich and Cantelo 1999).

Semiochemicals are used by many natural enemies to find their hosts (kairomones). Many of these relationships have been discovered as a result of isolation and synthesis of aggregation or sex pheromones of the host or prey pest species. When researching novel pheromones and candidate pheromones in the field, researchers should always be alert to occurence, behavior, and capture of non-target species, including predators and parasitoids. Among a number of examples, predaceous clerid beetles and hymenopterous parasitoids are attracted to bark beetle pheomones, where a semiochemical evolutionary race apparently occurs, based on selection for bark beetle volatiles which are less attractive to the natural enemies (Raffa and Dahlsten 1995; Raffa et al. 2007). Herbivorous and predaceous pentatomid bugs' male-produced aggregation pheromones are attractive to a variety of tachinid parasitoids (Aldrich et al. 2007).

So-called semiochemical eavesdropping poses significant non-target risks to natural enemies when pheromone-based attract-and-kill or mass trapping tactics are used against their pest hosts. Some of these systems might be altered chemically so as to discourage the natural enemies from attraction to the host pheromone, for instance by adding non-host-plant volatiles (Zhang and Schlyter 2010). By altering the blend, concentration, temporal pattern, or accompanying stimuli (e.g. visual design of traps or positioning of bait), the pheromone function might be preserved or enhanced, while the kairomonal function might be reduced or eliminated for natural enemies.

Host plants produce semiochemicals attractive to natural enemies of their herbivores, constituting synomones between the first and third trophic levels. Induced volatile synomones released by herbivore-damaged plants enable caterpillar parasitoids to find their hosts, a response with a strong learning component (Turlings et al. 1990). Entomopathogenic nematodes to infect their chrysomelid hosts feeding on maize roots underground (Rasmann et al. 2005). Crop plants may have lost this function in some cases, but cultivars can also be selected or even engineered for stronger attraction (constitutive or facultative) to biological controls (Köllner et al. 2008). The management implications of induced plant semiochemical signals are potentially valuable, but complex: for instance, parasitized herbivores feeding on plants attract hyperparasitoids (Poelman et al. 2012). Plant-derived natural enemy attractants can also be broadcast to attract natural enemies, although the advisability of such an approach has been debated, with possible negative effects on natural enemies and pollinators (Kaplan 2012), and varying effects depending on ecological context (Rodriguez-Saona et al. 2011). Constitutive emission of semiochemicals by plants may also prove ineffective due to lack of pulsing, or desensitation in target insects (e.g. Kunert et al. 2010).

Parasitoids and predators also use floral and other plant scents to locate non-prey food (Wäckers 2004; Lundgren 2009; Choate and Lundgren in press). Natural enemies rewarded in this way may increase protection of their plant food providers, thus participating in a more diffuse mutualism, or function to better suppress pests in adjacent crops. Plants produce specific attractants for natural enemies, sometimes seemingly deceptively, as in the case of orchids producing aphid alarm pheromones attractive to adult syrphids (Stökl et al. 2011).

Herbivores and other potential prey may sense and avoid their predators using olfactory cues, as in ovipositing mosquitoes avoiding predacious bugs (Silberbush et al. 2010). These avoidance interactions also occur among predators, to avoid intraguild predation and cannibalism through deposit and detection of inter- and intraspecific semiochemicals. Lacewings, for instance, avoid ovipositing in areas with lady beetle tracks (Chauhan and Weber 2008) and vice versa, and there is frequently more or less mutual avoidance also within aphidophagous predator taxa (Růžička 1997; Lucas 2005). Host-marking pheromones function in many parasitoids, as well as phytophagous insects, to spread oviposition over larger numbers of hosts, and this involves many complexities including super- and hyperparasitism (Nufio and Papaj 2001).

Semiochemical mimicry includes emission of another species' pheromone as an allomone for attraction then predation. Bolas spiders produce moth sex pheromones to lure male noctuids as prey, varying emissions among chemically diverse volatiles; there are probably many other examples of this strategy in spiders (Stowe et al. 1995). Such olfactory mimicry may also be used to repel, as in the case of phalangids and termites using ant alarm pheromones to repel predator ants (Howard and Akre 1995). Converse to this conspicuous semiochemical signalling is semiochemical use in crypsis (Ruxton 2009): for example, lepidoptera larvae may employ acquired chemical phytomimicry for crypsis to ants traversing twigs (Akino et al. 2004; Portugal and Trigo 2005). Or, predators may camouflage themselves to avoid detection by prey (e.g. reduviids among aphids) or prey defenders (typically ants) (Ruxton et al. 2005).

Social insects such as wasps and ants, are some of the most abundant predators in many ecosystems (Hölldobler and Wilson 1990). Their spectra of pheromones offer intriguing possibilities for manipulation, including focusing their potent consumption of prey on targets selected by pest managers.

There are additional roles of semiochemicals in nature, and for each role there are a number of potential applications to increase effectiveness of biological control for pest management. However, as in nature, there is no one effective solution: use of semiochemicals in concert with biological controls must take account of the complex ecosystems in which these mechanisms have evolved and are deployed.

## References

Akino, T., K. I. Nakamura, and S. Wakamura. 2004. Diet-induced chemical phytomimesis by twig-like caterpillars of *Biston robustum* Butler (Lepidoptera: Geometridae). Chemoecology 14: 165-174.

Aldrich, J. R. 1999. "Predators." pp. 357-383. *In* Hardie, J. and A.K. Minks (eds.), Pheromones of non-lepidopteran insects associated with agricultural plants. CABI Publishing, Wallingford, UK.

Aldrich, J. R., and W. W. Cantelo. 1999. Suppression of Colorado potato beetle infestation by pheromone-mediated augmentation of the predatory spined soldier bug, *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae). Agric. For. Entomol. 1: 209-217.

Aldrich, J. R., A. Khrimian, and M.J. Camp. 2007. Methyl 2,4,6-decatrienoates attract stink bugs and tachinid parasitoids. J. Chem. Ecol. 33: 801–815.

Chauhan, K. R., and D.C. Weber. 2008. Lady beetle (Coleoptera: Coccinellidae) tracks deter oviposition by the goldeneyed lacewing, *Chrysopa oculata*. Biocontrol Sci. Technol. 18: 727-731.

Chauhan, K. R., Q.-H. Zhang, and J. R. Aldrich. 2004. Iridodials: Enantiospecific synthesis and stereochemical assignment of the pheromone for the goldeneyed lacewing, *Chrysopa oculata* (Neuroptera: Chrysopidae). Tetrahedron Lett. 45: 3339-3340.

Chauhan, K. R., V. Levi, Q.-H. Zhang, and J. R. Aldrich. 2007. Female goldeneyed lacewings (Neuroptera: Chrysopidae: *Chrysopa oculata*) approach but seldom enter traps baited with the male-produced compound, iridodial. J. Econ. Entomol. 100: 1751-1755.

Choate, B. A., and J. G. Lundgren. 2013. Why eat extrafloral nectar? Understanding food selection by *Coleomegilla maculata* (Coleoptera: Coccinellidae). Biocontrol, *in press*.

Greenstone, M. H., and J. C. Dickens. 2005. "The production and appropriation of chemical signals among plants, herbivores and predators." pp. 139-165. *In* Barbosa, P. and I. Castellanos, (eds.), Ecology of predator-prey interactions. Oxford University Press, Oxford, UK.

Hölldobler, B., and E.O. Wilson. 1990. The Ants. Belknap Press, 732 pp.

Howard, R. W., and R.D. Akre. 1995. Propaganda, crypsis and slavemaking. pp. 364-429. *In* R. T. Cardé and W.J. Bell (eds.), Chemical ecology in insects 2. Chapman and Hall, London.

Kaplan, I. 2012. Attracting carnivorous arthropods with plant volatiles: The future of biocontrol or playing with fire? Biol. Control 60: 77-89.

Köllner, T. G., M. Held, C. Lenk, I. Hiltpold, T. C. Turlings, J. Gershenzon, and J. Degenhardt. 2008. A maize (E)-β-caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. Plant Cell Online 20: 482-494.

Kunert, G., C. Reinhold, and J. Gershenzon. 2010. Constitutive emission of the aphid alarm pheromone, (E)-β-farnesene, from plants does not serve as a direct defense against aphids. BMC Ecol. 10: 23.

Lucas, É. 2005. Intraguild predation among aphidophagous predators. Eur. J. Entomol, 102: 351-364.

Lundgren, J. G. 2009. Relationships of natural enemies and non-prey foods. Springer, London, UK, 453 pp.

Nufio, C. R., and D. R. Papaj. 2001. Host marking behavior in phytophagous insects and parasitoids. Entomol. Exp. Applic. 99: 273–293

Poelman, E. H., M. Bruinsma, F. Zhu, B. T. Weldegergis, A. E. Boursault, Y. Jongema, J. J. A. van Loon, L. E. M. Vet, J. A. Harvey., and M. Dicke. 2012. Hyperparasitoids use herbivore-induced plant volatiles to locate their parasitoid host. PLOS Biology 10: e1001435.

Portugal, A. H. A., and J. R. Trigo. 2005. Similarity of cuticular lipids between a caterpillar and its host plant: a way to make prey undetectable for predatory ants? J. Chem. Ecol. 31: 2551-2561.

Raffa, K. F., and Dahlsen, D. L. 1995. Differential responses among natural enemies and prey to bark beetle pheromones. Oecologia 102: 17–23.

Raffa, K. F., K. R. Hobson, S. LaFontaine, and B. H. Aukema. 2007 Can chemical communication be cryptic? Adaptations by herbivores to natural enemies exploiting prey semiochemistry. Oecologia 153: 1009–1019.

Rasmann, S., T. G. Köllner, J. Degenhardt, I. Hiltpold, S. Toepfer, U. Kuhlmann, J. Gershenzon, and T. C. J. Turlings. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434: 732-737.

Rodriguez-Saona, C., I. Kaplan, J. Braasch, D. Chinnasamy, and L. Williams. 2011. Field responses of predaceous arthropods to methyl salicylate: a meta-analysis and case study in cranberries. Biol. Control 59: 294-303.

Ruxton, G. D. 2009. Non-visual crypsis: a review of the empirical evidence for camouflage to senses other than vision. Philos. Trans. R. Soc. B. 364: 549-557.

Ruxton, G. D., T. N. Sherratt, and M. P. Speed. 2005. Avoiding attack: The evolutionary ecology of crypsis, warning signals and mimicry. Oxford University Press, 264 pp.

Růžička, Z. 1997. Recognition of oviposition-deterring allomones by aphidophagous predators (Neuroptera: Chrysopidae, Coleoptera: Coccinellidae). Eur. J. Entomol. 94: 431-434.

Silberbush, A., S. Markman, E. Lewinsohn, E. Bar, J. E. Cohen, and L. Blaustein. 2010. Predator-released hydrocarbons repel oviposition by a mosquito. Ecol. Lett. 13: 1129-1138.

Stökl, J., J. Brodmann, A. Dafni, M. Ayasse, and B.S. Hansson. 2011. Smells like aphids: orchid flowers mimic aphid alarm pheromones to attract hoverflies for pollination. Proc. Roy. Soc. B. 278: 1216-1222.

Stowe, M. K., T. C. Turlings, J. H. Loughrin, W. J. Lewis, and J. H.Tumlinson. 1995. The chemistry of eavesdropping, alarm, and deceit. Proc. Natl Acad. Sci. 92: 23-28.

Turlings, T. C. J., J. H. Tumlinson. and W. J. Lewis. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250: 1251–1253.

Wäckers, F. L. 2004. Assessing the suitability of flowering herbs as parasitoid food sources: flower attractiveness and nectar accessibility. Biol. Control. 29: 307-314.

Witzgall, P., P. Kirsch, and A. Cork. 2010. Sex pheromones and their impact on pest management. J. Chem. Ecol. 36: 80-100.

Zhang, Q. H., and F. Schlyter. 2010. Inhibition of predator attraction to kairomones by non-host plant volatiles for herbivores: a bypass-trophic signal. PloS One 5: e11063.

# Plant volatiles for monitoring natural enemy activity and phenology

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Modern agricultural systems have an impressive record of productivity, but this has been achieved through increased intensification and heavy reliance on agricultural inputs some of which have had unintended negative consequences for sustainability and environmental health. As a result, it is generally recognized that greater ecological sustainability is an increasingly important goal for the future of agricultural systems (Kornegya and Harwood 2010; Gomiero et al. 2011). Consequently, this has raised awareness of the value of natural ecosystem services in agriculture (Power 2010), such as biological control, which can directly substitute for pesticides in the development of more sustainable pest management practices (Shennan 2008; Ekström and Ekbom 2011).

Although the value of biological pest control has been well documented for over a century (e.g. DeBach 1974; Van Driesche et al. 2008), it has yet to be more widely adopted and implemented in modern agricultural systems as a central component of pest management. One of the key barriers to more widespread adoption of biological control is our inability to readily detect and monitor the activity of natural enemies in our crops. While pheromones have been widely implemented for monitoring the abundance and seasonal activity of a variety of crop pests since the mid 1980s (Witzgall et al. 2010), we have been slow to develop new tools to aid pest management decisions with regard to monitoring the abundance and activity of natural enemies. Sampling for natural enemies is still based on labor intensive approaches, such as host rearing to estimate parasitism and either sweep nets or beating trays to estimate predator abundance (Mills 2005), methods that are impractical for making pest management decisions. While certain groups of parasitoids are known to be attracted to the pheromones used by their hosts or prey (Zuk and Kolluru 1998), a far greater range of predators and parasitoids respond to plant volatiles (Mumm and Dicke 2010; Hare 2011). There has been no shortage of interest in the potential for utilizing plant volatiles for the enhancement of natural enemy activity in crops (Turlings and Ton 2006; Khan et al. 2008), but caution is needed, as there are many unanswered questions that need to be addressed before these chemical signals can be successfully implemented as a component of conservation biological control (Gurr and Kvedaras 2010; Jones et al. 2011; Kaplan 2012).

In contrast, however, plant volatiles offer more immediate opportunities for monitoring rather than manipulation of natural enemy populations in agricultural crops. Plant volatile traps offer a practical monitoring tool for assessing the abundance and seasonal activity of natural enemies in agricultural crops. The traps are both simple and familiar enough to be adopted by field scouts to further inform pest management decisions. A variety of individual plant volatiles have been tested as attractants for natural enemies (James 2005; Kaplan 2012), but there is increasing evidence that stronger responses are generated by volatile blends (Jones et al. 2011; McCormick et al. 2012). As part of a large, multi-state, project we have been investigating the use of plant volatile traps for monitoring both the activity and the seasonal phenology of natural enemies in tree fruit and nut orchards in the western U.S. (Jones et al. 2009).

With a view to developing a practical lure that can be used to monitor a broad range of natural enemy taxa as a tool for visualizing natural enemy activity and the impact of pest management choices on natural enemy populations in tree fruit and nut crops, we have field tested a series of 14 different plant volatiles. The plant volatile lures were enclosed within 3 x 5cm polyethylene tubing bags with both volume of the attractant and thickness of the tubing adjusted to match the relative volatility of the attractants and to provide a constant release rate over a period of one month. Those plant volatiles that have a broader spectrum of attractiveness to natural enemies, such as acetic acid (AA), acetophenone (AP), geraniol (GER), methyl salicylate (MS), and 2-phenylethanol (PE) have been field tested either individually or as two, three or four component blends (each component in a separate polyethylene bag). The lures were field tested by placement either within white delta traps, or above yellow sticky cards. Our field tests have been replicated in apple and cherry orchards in Washington state, pear and cherry orchards in Oregon, and in walnut orchards in California to determine consistency of natural enemy responses to the different lures.

Volatile blends almost always proved to be more attractive than single volatile components, but different blends proved to be more attractive to different natural enemy taxa. Thus, for example, the best lure for green lacewings, such as *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), was a blend of AA+MS+PE, but it is interesting to note that the inclusion of AA in this blend reduced the attractiveness of the lures for brown lacewings *Hemerobius* spp. (Neuroptera: Hemerobiidae) and hoverflies, such as *Eupeodes fumipennis* (Thoms.) (Diptera: Syrphidae). The best lures for these two latter groups were AP+PE and GER+PE respectively. In general, the three component blend GER+MS+PE performed well as a lure for a variety of different natural enemy taxa, highlighting the rich diversity of natural enemies present in tree crops in the western U.S. Further, the use of plant volatile blends highlighted the need to focus on indicator species for the development of plant volatile traps as a practical monitoring tool. Additional analysis is currently in progress to select readily identifiable natural enemy species or natural enemy species groups that can best serve as indicators of the compatibility of a crop environment for the provision of biological control services.

We have also used plant volatile traps baited with either single component (such as squalene) or multi-component blend (GER+MS+PE) lures for season-long monitoring of the phenology of natural enemy flights. While degree-day phenology models are frequently used to predict the seasonal flights of pest species, little attention has been paid to the development of similar phenology models for natural enemies, despite their practical value in identifying when beneficial taxa would be most vulnerable to applications of insecticides. Season-long monitoring of natural enemies in plant volatile traps has allowed us to develop degree-day phenology models for two species of lacewing species (*Chrysopa nigricornis* Burmeister and *Chrysoperla carnea*), a syrphid fly (*Eupeodes fumipennis*), and the mirid bug, *Deraeocoris brevis* (Uhler) (Hemiptera: Miridae). By knowing if the timing of pest management interventions for key pests broadly overlap with the flights of important natural enemies of secondary pests in a crop, a grower can be better informed as to whether to choose a more selective control strategy in place of a less selective insecticide.

The preliminary results from our large scale project in tree fruit and nut crops in the western U.S. are very promising and show considerable potential for the development of simple and effective monitoring tools for natural enemies. Plant volatile traps offer a new opportunity for pest managers to better visualize the activity and phenology of biological control services in agricultural crops, which once integrated into the pest manager's toolkit should lead to greater sustainability of pest management programs.

## References

DeBach, P. 1974. Biological control by natural enemies. Cambridge University Press, Cambridge, UK.

Ekström, G., and B. Ekbom. 2011. Pest control in agro-ecosystems: An ecological approach. Crit. Rev. Plant Sci. 30: 74-79.

Gomiero, T., D. Pimentel, and M. G. Paoletti. 2011. Is there a need for a more sustainable agriculture? Crit. Rev. Plant Sci. 30: 6-23.

Gurr, G. M., and O. L., Kvedaras. 2010. Synergizing biological control: scope for sterile insect technique, induced plant defences and cultural techniques to enhance natural enemy impact. Biol. Control 52: 198–207.

Hare, J. D. 2011. Ecological role of volatiles produced by plants in response to damage by herbivorous insects. Annu. Rev. Entomol. 56: 161–180.

James, D. G. 2005. Further field evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. J. Chem. Ecol. 31: 481–495.

Jones, V. P., T. R. Unruh, D. R. Horton, N. J. Mills, J. F. Brunner, E. H. Beers, and P. W. Shearer. 2009. Tree fruit IPM programs in the western United States: the challenge of enhancing biological control through intensive management. Pest Man. Sci. 65: 1305–1310.

Jones, V.P., S. A. Steffan, N. G. Wiman, D. R. Horton, E. Miliczky, Q.-H. Zhang, and C. C. Baker. 2011. Evaluation of herbivore-induced plant volatiles for monitoring green lacewings in Washington apple orchards. Biol. Control 56: 98–105.

Kaplan I. 2012. Attracting carnivorous arthropods with plant volatiles: the future of biocontrol or playing with fire? Biol. Control, 60: 77-89.

Khan, Z. R., D. G. James, C. A. O. Midega, and J. A., Pickett. 2008. Chemical ecology and conservation biological control. Biol. Control 45: 210–224.

Kornegya, J., and R. Harwood. 2010. Towards sustainable agricultural systems in the 21st Century. National Academy of Sciences, Washington, USA.

McCormick, A.C., S. B. Unsicker, and J. Gershenzon. 2012. The specificity of herbivore-induced plant volatiles in attracting herbivore enemies. Trends Plant Sci. 17: 303-310.

Mills, N. J. 2005. Parasitoids and predators. In: Insect sampling in forest ecosystems. S. R. Leather (ed.), Blackwell, Oxford, UK, pp. 254-278.

Mumm, R., and M. Dicke. 2010. Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. Canadian J. Zoolog. 88: 628–667.

Power, A. G. 2010. Ecosystem services and agriculture: tradeoffs and synergies. Philos. Trans. R. Soc. B. 365: 2959–2971.

Shennan, C. 2008. Biotic interactions, ecological knowledge and agriculture. Philos. Trans. R. Soc. B. 363: 717–739.

Turlings, T. C. J., and J. Ton. 2006. Exploiting scents of distress: the prospect of manipulating herbivore-induced plant odours to enhance the control of agricultural pests. Curr. Opin. Plant Biol. 9: 421–427.

Van Driesche, R. G., M. S. Hoddle, and T. Center. 2008. Control of pests and weeds by natural enemies, Blackwell, Malden, USA

Witzgall, P., P. Kirsch, and A. Cork. 2010. Sex pheromones and their impact on pest management. J. Chem. Ecol. 36: 80–100.

Zuk, M., and G. R. Kolluru. 1998. Exploitation of sexual signals by predators and parasitoids. Q. Rev. Biol. 73: 415-438.

# Field application of semiochemicals for parasitoids management

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Nowadays one of the main points of pest management sciences is to minimize or eliminate the use of pesticides, and to improve the quality and productivity of food. These paradigms aim to develop a more sustainable agricultural system. The approach proposed for pest management is one where the pests, diseases and weeds are integrated using different practices, where the uses of existent biodiversity (biological control) have a central role. Success of biological control depends of two important factors: 1) attraction of the natural enemy in synchronization with the pest population, and 2) to retain them in the field (Vinson 1985). To achieve these objectives, the application of semiochemicals, as chemicals cues, to manipulate the behaviour of natural enemies has been presented as one of the possible alternatives. For parasitoids, foraging behaviour includes several sequential steps, such as: habitat location, host selection, host recognition, host suitability and oviposition (Godfray 1994). During these steps, natural enemies can use physical, biochemical and, mainly, semiochemicals cues (Vinson 1985; Godfray 1994). Identification of the cues used by parasitoids in each step may be relevant to select and obtain semiochemicals to be used for their behaviour manipulation. Source of semiochemicals for parasitoids are diverse and could include volatile organic compounds (VOCs) released by plants, which includes the herbivore and oviposition induced plant volatiles or constitutive volatile organic compounds that could be used by parasitoids for long distance orientation (Dicke 1994; Heil 2008; Moraes et al. in press). In a similar way parasitoids can use pheromones and other semiochemicals as cues from hosts, or from habitat or microhabitat components (Moraes et al. in press). Both conservative and augmentative biological control techniques offer opportunities to use semiochemicals to improve/aid the efficiency of the management practices. Different strategies are been developed to the use semiochemicals in the field, which can act directly on parasitoid behaviour, or indirectly, inducing the plant production and liberation of VOCs, that are attractive to parasitoids. Other strategy include the combination of semiochemicals with the manipulation of the environment, as for example use of plants as food rewards for natural enemies in order to improve their efficiency after been attracted to selected areas by semiochemicals ("attract and reward" strategy) (Simpson et al. 2011), or the use of plant semiochemicals in push-pull systems, that involve the behavioural manipulation of pests and natural enemies using different semiochemicals stimuli from plants or synthetic semiochemicals in traps (Khan et al. 2011). In Brazil phytophagous stink bugs (Hemiptera: Pentatomidae), as the brow stink bug *Euschistu heros (Fabr.)*, the green stink bug, *Nezara viridula* (L.) and the red banded stink bug *Piezodorus guildinii* (Westwood) are the main pests on soybean and other grains crop and egg parasitoids are their main natural enemies, specially *Telenomus podisi* (Ashmead) and *Trissolcus basalis* (Wollaston) (Borges *et al*. 2003; Laumann *et al*. 2008). Difficulties to mass rear egg parasitoids and their high dispersion rate from the release areas become this technology, too laborious although very promising. Thus, in order to improve the efficiency of the augmentative release of egg parasitoids, or to develop conservative biological control, behavioural manipulation of parasitoid using semiochemicals could be a practical solution to attract and retain them in the field (Borges and Aldrich 1994). Ina field experiment that lasted one week, slow release of green leaf volatiles, such as the aldehyde (*E*)-2-hexenal, (a compound present on methatoracic glands of different species of stink bugs), increased the number of parasitoids and the parasitism of eggs naturally laid by caged females in the treated areas. In another experiment, a full crop season on a more extensive area (soybean plots of 400 $m^2$) with applications of four septa/plot impregnated with (*E)*-2-hexenal at two different doses (4.0 mg and 10.0 mg) showed that the abundance of parasitoids did not differ from control plots (Laumann et al. 2007). In the (*E*)-2-hexenal treated plots, recruitment of parasitoids started in early phenological stage of the crop. Our results showed that the dosage of semiochemicals and the density of dispensers need to be re-evaluated to both attract and retain the egg parasitoids in the treated areas. The use of *cis*-jasmone, a phytohormone that induces indirect defences in wheat and soybean (Moraes et al. 2009), was studied in field experiments in small plots (2.0 $m^2$). Soybean plants were prayed with a solution of *cis*-jasmone (6 ml of a solution of 0.25 g *cis*-jasmone + 0.1 g de Tween20, in 1.0 l of water) in two areas, one within a soybean and another within a *Crotalaria* matrix. The parasitoid community was sampled weekly with yellow sticky traps (n = 3/plot). Parasitism was monitored with sentinel eggs of *E. heros* (n = 150/plot). Stink bugs populations were monitored weekly by sampling each plot with shake-cloth technique. The application of *cis*-jasmone in the field did not significantly affect the parasitoid community but the treatment with *cis*-Jasmone enhanced the Platygastridae population, principally of *Trissolcus* spp. and *Telenomus* spp. but had no effect on the occurrence and intensity of parasitism and in the number of stink bugs (Vieira et al 2012). Our results showed that field application of semiochemicals in soybean cultures has the potential to be used in the behavioural manipulation of egg parasitoids. Notwithstanding the use of semiochemicals in soybean and others crops needs to be linked with the redesign of some agronomic practices, including reorganization of landscape with restoration/maintenance of native areas, reduction of pesticide use and the incorporation of new production technologies more beneficial to natural enemies.

Acknowledgments: To CNPq, FAP-DF and Embrapa for financial support.

## **References**.

Borges, M., and J. R. Aldrich. 1994. Estudos de semioquímicos para o manejo de Telenominae. Anais Socied. Entomol. Brasil. 23: 575–577.

Borges, M., S. Colazza, P. Ramirez-Lucas, K. R. Chauhan, J. R. Aldrich, and M. C. B. Moraes. 2003. Kairomonal effect of walking traces from *Euschistus heros* (Heteroptera: Pentatomidae) on two strains of *Telenomus podisi* (Hymenoptera: Scelionidae). Physiol. Entomol. 28:349-355.

Dicke, M. 1994. Why do plants “talk”? Chemoecology, 5/6: 159–165

Godfray, H. C. J. 1994. Parasitoids, Behavioural and Evolutionary Ecology. Princeton University Press, Princeton, New Jersey. 473 pp.

Heil, M. 2008. Indirect defence via tritrophic interactions. New Phytol. 178: 41–61.

Khan, Z.; Midega, C.; Pittchar, J., Pickett, J., Bruce, T. 2011. Push–Pull Technology: A conservation agriculture approach for integrated management of insect pests, weeds and soil health in Africa UK Government’s foresight food and farming futures project. Int. J. Agric. Sustainability. 9: 162–170.

Laumann, R. A., M. C. B. Moraes, M. Pareja, G. C. Alarcão, A. C. Botelho, A. H. N. Maia, E. Leonardez, and M. Borges. 2008. Comparative biology and functional response of *Trissolcus* spp. (Hymenoptera: Scelionidae) and implications for stink bugs (Hemiptera: Pentatomidae) biological control. Biol. Control 44: 32–41.

Laumann, R. A. , M. C. B. Moraes, M. B. Corrêa Ferreira, L. D. Caetano, A. R. A. Vieira, C. S. S. Pires, and M. Borges. 2007. Field applications of (*E*)-2-hexenal: a kairomone for egg parasitoids (Hymenoptera: Scelionidae). *In* 23rd Annual Meeting of the International Society of Chemical Ecology, 2007, Jena. Book of Abstracts, p. 177.

Moraes, M. C. B., M. Borges, and R.A. Laumann. 2013. Application of chemical cues in arthropod pest management for arable crops. *In* E. Wajnberg and S. Colazza (Eds.), Chemical Ecology of Insect Parasitoids. Wiley-Blackwell. In press.

Simpson, M., G. M. Gurr, A. T. Simmons, S. D. Wratten, D. G James, G. Leeson, H. I. Nicol, and G. U. S. Orre-Gordon. 2011. Attract and reward: combining chemical ecology and habitat manipulation to enhance biological control in field crops. J. Appl. Ecol. 48: 580–590.

Vinson, S. B. 1985. The behaviour of parasitoids, pp. 417–469. *In* G.A. Kerkut and L.I. Gilbert. Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon Press, New York.

Vieira, C. R., M. C. B. Moraes, M. Borges, E. R. Sujii, and R. A. Laumann, 2012. cis-Jasmone indirect action on egg parasitoids (Hymenoptera: Scelionidae) and its application in biological control of soybean stink bugs (Hemiptera: Pentatomidae). Biological Control, http://dx.doi.org/10.1016/j.biocontrol.2012.10.004.

# Response of insect predators to methyl salicylate in cranberries

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Agricultural practices to conserve natural enemies of herbivorous pests are desirable in biological control. An approach towards this is the manipulation of plant volatiles to attract predators and parasitoids of herbivores. Most plants emit a unique blend of volatile organic compounds (VOCs) that attract natural enemies of herbivores, and emissions of these VOCs often increase in response to herbivore feeding, thus providing natural enemies with highly detectable and reliable information on the location (and possible identity) of their prey or hosts. In the last decade, the potential of using synthetic VOCs to attract predaceous arthropods in agricultural fields has been investigated. In particular, methyl salicylate (MeSA) has recently been used in multiple studies to attract natural enemies of insect pests in agricultural fields (e.g. James 2003; James 2005; Lee et al. 2010; Mallinger et al. 2011). Induced by herbivore feeding, this VOC is a component of many leaf and floral blends, that can serve as a volatile signal in triggering induced defenses in plants, and is commercially available to growers as a slow-release dispenser (PredaLure; AgBio Inc., Westminster, Colorado) for the attraction of natural enemies of agricultural pests.

In 2008, I tested the attraction of insect predators to MeSA lures (PredaLure) in commercial cranberry bogs in New Jersey (USA). The response of insect predators to PredaLure was monitored weekly from bloom until fruit maturation. All bogs were located within a single cranberry farm and contained a pair of yellow sticky traps: one baited with PredaLure and one unbaited (no lure control). MeSA-baited traps caught greater numbers of adult hoverflies (mainly *Toxomerus marginatus*) (Diptera: Syrphidae), lady beetles (in particular *Coccinella septempunctata*) (Coleoptera: Coccinellidae), and green lacewings (Neuroptera: Chrysopidae) compared with unbaited traps. Subsequent studies were conducted in 2011-2012 to evaluate the attraction of predators to PredaLure throughout the growing season (April-August), and to determine if attraction of predators to PredaLure leads to higher egg predation. These experiments were done in eight cranberry bogs from four commercial farms. Each bog contained a PredaLure-baited and an unbaited yellow sticky trap. Egg masses of the European corn borer were placed in eight additional bogs near PredaLure baits or unbaited controls. Predator abundance and egg predation were monitored twice a month from May until August. Results from these experiments will be presented and discussed.

To determine the attractiveness of MeSA to predators over various distances, I conducted a field experiment where predator abundance was monitored using traps placed near the PredaLure (0 m), as well as at 2.5, 5, and 10 m away from the lure. Adult *T. marginatus*, the dominant predator species, showed a clear attraction to the point source but not to the other distances. In complementary studies, I determined the temporal pattern of PredaLure emission rate, the source of MeSA emissions in cranberries, and the volatile response of cranberry leaves to PredaLure exposure. I showed that MeSA emissions from PredaLures dropped quickly during the first week of deployment in the field but remained relatively high for over four weeks. Flowering, but not vegetative, vines were a primary source of MeSA in cranberries and, coincidently, adult predators were most active during bloom. However, exposure to PredaLures triggered elevated MeSA emissions from vegetative vines. In conclusion, insect predators were broadly attracted to MeSA in cranberries. Furthermore, this study is the first to demonstrate that PredaLure exposure can elevate MeSA emissions in cranberries. Parts of this work have been published in Rodriguez-Saona et al. (2011).

## References

James, D. G. 2003. Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing, *Chrysopa nigricornis*. J. Chem. Ecol. 29: 1601–1609.

James, D. G. 2005. Further field evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. J. Chem. Ecol. 31: 481–495.

Lee, J. C. 2010. Effect of methyl salicylate-based lures on beneficial and pest arthropods in strawberry. Environ. Entomol. 39: 653–660.

Mallinger, R. E., D. B. Hogg, and C. Gratton. 2011. Methyl salicylate attracts natural enemies and reduces populations of soybean aphids (Hemiptera: Aphididae) in soybean agroecosystems. J. Econ. Entomol. 104: 115–124.

Rodriguez-Saona, C., I. Kaplan, J. Braasch, D. Chinnasamy, and L. Williams. 2011. Field responses of predaceous arthropods to methyl salicylate: A meta-analysis and case study in cranberries. Biol. Control. 59: 294–303.

# Smells of the dark: prospects of exploiting belowground chemical ecology for biological pest control

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Chemical ecology has historically focused on aboveground systems (Hartmann 2007), but recently considerable attention has shifted to chemically mediated interactions that occur belowground (e.g. Boff et al. 2001; Boff et al. 2002; Mathesius et al. 2003; Callaway et al. 2004; Rasmann et al. 2005; Ali et al. 2010; Rasmann et al. 2011). As primary producers, plants play a major role in these interactions (Rasmann et al. 2012) by exuding up to 20% of their photosynthetic fixed carbon via belowground tissues (Barber and Martin 1976).

In North America, a little less than one fifth of all insect families contain insect species that feed on plant roots (Rasmann and Agrawal 2008), and in agricultural systems, they can cause tremendous economic losses. But soil-dwelling insect have first to locate roots before they harm this important plant tissue and it has become evident that they rely on volatile cues to localize their host plants. Carbon dioxide is the simplest and certainly the most ubiquitous volatile present in soil that indicates the presence of life, and it has long erroneously been thought to be a major cue belowground (Johnson and Gregory 2006; Johnson and Nielsen 2012 and references therein). In a recent comprehensive review, Wenke et al. (2010) made an inventory of a wide range of volatiles, which soil-dwelling insect pests use to locate their host plants. We envision that a good understanding of this crucial step in the insects' lifecycle can be exploited to lure them away from plants before their establishment on the roots. Based on this notion and after consulting the available literature, Hiltpold et al. (2012) formulated an artificial blend of attractants in order to lure larval stages of the western corn rootworm (WCR) *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) towards capsules containing entomopathogenic nematodes (EPN). WCR is a major pest of maize in North America and Europe (Gray et al. 2009; Wesseler and Fall 2010; Mitchell 2011) and can potentially be controlled by insect-killing nematodes (Toepfer et al. 2009). The attraction of WCR larvae to the capsules was successful in laboratory experiments, where as many larvae were attracted towards capsules coated with the synthetic blend of attractants as towards the root system of a maize plant (Hiltpold et al. 2012). Despite these promising laboratory results, field trials did not show the anticipated synergistic effect of the attractive capsule coating on the reduction of root damage, although EPN encapsulation *per se* was found to be an effective way to apply these biological control agents in the field (Hiltpold et al. 2012). As also shown by others (e.g. Hibbard et al. 1995), manipulating belowground chemical ecology to ensure a better crop protection has great potential, but further fine tuning is still needed (Hiltpold and Turlings 2012).

Damaged roots have been shown to emit volatiles that recruit EPN both in agricultural and natural ecosystems (Hiltpold et al. 2010a; Ali et al. 2011; Rasmann et al. 2011). The first such belowground "call for help" that was identified is the sesquiterpene (*E*)-β-caryophyllene (EβC) (Rasmann et al. 2005). This compound is emitted by maize roots fed on by WCR larvae and is attractive to the EPN *Heterorhabditis megidis* Poinar, Jackson & Klein (Rhabditida: Heterorhabditidae) in the laboratory, as well as in the field (Rasmann et al. 2005). However, not all maize varieties produce the same belowground signals (Erb et al. 2011), and most tested

American maize lines no longer emit EβC (Köllner et al. 2008). Because WCR larvae themselves also show attraction to this compound (Robert et al. 2012), it has been hypothesized that this trait had unconsciously been bred out while selecting for WCR resistance (Köllner et al. 2008; Robert et al. 2012). Through genetic manipulation, the EβC signal could be restored in a non-emitting maize line (Degenhardt et al. 2009). Subsequent field trials with these lines and isogenic equivalents showed that the restored ability to recruit EPN resulted in a significant reduction of WCR damage in the genetically modified plants (Degenhardt et al. 2009). This was the first demonstration of a perfect compatibility between two approaches that are normally considered to be diametrically opposed, biological control versus the use of genetically engineered plants (Degenhardt et al. 2009). To better exploit this induced defense in maize Hiltpold et al. (2010c) selected a strain of the EPN *H. bacteriophora* Poinar (Rhabditida: Heterorhabditidae) for high responsiveness to EβC. This nematode species exhibited high effectiveness in killing WCR larval stages (Kurzt et al. 2009), but only a weak response to EβC (Hiltpold et al. 2010a). The selection resulted in a strain responding much to the alarm signal and, again field trials revealed it to be better at controlling WCR population when used with an EβC-emitting variety of maize (Hiltpold et al. 2010c, b). Emissions of EPN-attracting volatiles by herbivore-damaged roots have been found for a diverse range of plant species (Boff et al. 2001; van Tol et al. 2001; Ali et al. 2010, 2011; Turlings et al. 2012), but these nematodes are also showing positive chemotaxis to various insect-host cues (Dillman et al. 2012). Each of these (sometimes not yet) identified compounds offers the same potential as EβC for exploitation in sustainable pest control (Hiltpold and Turlings 2012).

Among the belowground pests we can also find numerous plant-parasitic nematodes. Those nematodes too respond to chemical cues emitted by roots and good knowledge of the chemical ecology underlying these interactions could lead to the development of novel control techniques against plant parasitic nematodes. For instance, Tanino et al. (2011) synthetized an egg-hatching stimulating factor originally emitted by plant roots and that nematodes use to synchronize their egg hatching with plant phenology (Masamune et al. 1982; Mulder et al. 1992; Schenk et al. 1999), again opening the way to new management strategies of such belowground pests. In a recent study, Kaplan et al. (2012) identified ascaroside blends as important signals triggering dispersion in plant-parasitic nematodes, which could also be exploited.

The more we understand about belowground ecology, the more it is clear that it is extremely complex and a lot remains to be done not only in the field of chemical ecology, but also to understand the basic biology of the involved organisms (Cheeke et al. 2012). But as Ali et al. (2012) show, it is not necessary to know all the details of an interaction to already benefit from our knowledge in belowground chemical ecology. Thanks to new behavioral and analytical tools, some major issues in belowground chemical ecology will be soon elucidated and innovative applications for sustainable pest control will certainly result from these breakthroughs in soil ecology.

## References

Ali, J. G., H. T. Alborn, and L. L. Stelinski. 2010. Subterranean herbivore-induced volatiles released by citrus roots upon feeding by *Diaprepes abbreviatus* recruit entomopathogenic nematodes. J. Chem. Ecol. 36: 361–368.

Ali, J. G., H. T. Alborn, and L. L. Stelinski. 2011. Constitutive and induced subterranean plant volatiles attract both entomopathogenic and plant parasitic nematodes. J. Chem. Ecol. 99: 26–35.

Ali, J. G., H. T. Alborn, R. Campos-Herrera, F. Kaplan, et al. 2012. Subterranean, herbivore-induced plant volatile increases biological control activity of multiple beneficial nematode species in distinct habitats. PLoS One 7: e38146. doi:10.1371/journal.pone.0038146

Barber, D. A., and J. K. Martin. 1976. The release of organic substances by cereal roots into soil. New Phytol. 76: 60–80.

Boff, M. I. C., F. C. Zoon, and P. H. Smits. 2001. Orientation of *Heterorhabditis megidis* to insect hosts and plant roots in a Y-tube sand olfactometer. Entomol. Exp. Applic. 98: 329–337.

Boff, M. I. C., R. van Tol, and P. H. Smits. 2002. Behavioural response of *Heterorhabditis megidis* towards plant roots and insect larvae. Biocontrol 47: 67-83.

Callaway, R. M., G. C. Thelen, A. Rodriguez, and W. E. Holben. 2004. Soil biota and exotic plant invasion. Nature 427: 731–733.

Cheeke, T. E., D. C. Coleman, and D. H. Wall. 2012. Microbial ecology in sustainable agroecosystems, CRC Press, Boca Raton, FL, U.S.A.

Degenhardt, J., I. Hiltpold, T. G. Köllner, M. Frey, et al. 2009. Restoring a maize root signal that attracts insect-killing nematodes to control a major pest. P. Natl. Acad. Sci. USA. 106: 13213–13218.

Dillman, A. R., M. L. Guillermin, J. Lee, B. Kim, et al. 2012. Olfaction shapes host-parasite interactions in parasitic nematodes. P. Natl. Acad. Sci. USA. 109: E2324–E2333.

Erb, M., D. Balmer, E. S. De Lange, G. Von Merey, et al. 2011. Synergies and trade-offs between insect and pathogen resistance in maize leaves and roots. Plant Cell Environ. 34: 1088–1103.

Gray, M. E., T. W. Sappington, N. J. Miller, J. Moeser, et al. 2009. Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. Annu. Rev. Entomol. 54: 303–321.

Hartmann, T. 2007. From waste products to ecochemicals: Fifty years research of plant secondary metabolism. Phytochemistry 68: 2831–2846.

Hibbard, B. E., F. B. Peairs, S. D. Pilcher, M. E. Schroeder, et al. 1995. Germinating corn extracts and 6-methoxy-2-benzoxazolinone - western corn rootworm (Coleoptera, Chrysomelidae) larval attractants evaluated with soil insecticides. J. Econ. Entomol. 88: 716–724.

Hiltpold, I., and T. C. J. Turlings. 2012. Manipulation of chemically mediated interactions in agricultural soils to enhance the control of crop cests and to improve crop yield. J. Chem. Ecol. 38: 641–650.

Hiltpold, I., S. Toepfer, U. Kuhlmann, and T. C. J. Turlings. 2010a. How maize root volatiles influence the efficacy of entomopathogenic nematodes against the western corn rootworm? Chemoecology 20: 155–162.

Hiltpold, I., M. Baroni, S. Toepfer, U. Kuhlmann, et al. 2010b. Selective breeding of entomopathogenic nematodes for enhanced attraction to a root signal did not reduce their establishment or persistence after field release. Plant Signal. Beha. 5: 1450–1452.

Hiltpold, I., M. Baroni, S. Toepfer, U. Kuhlmann, et al. 2010c. Selection of entomopathogenic nematodes for enhanced responsiveness to a volatile root signal helps to control a major root pest. J. Exp. Biol. 213: 2417–2423.

Hiltpold, I., B. E. Hibbard, D. W. Freckman, and T. C. J. Turlings. 2012. Capsules containing entomopathogenic nematodes as a Trojan horse approach to control the western corn rootworm. Plant and Soil 358: 11–25.

Johnson, S. N., and P. J. Gregory. 2006. Chemically-mediated host-plant location and selection by root-feeding insects. Physiol. Entomol. 31: 1–13.

Johnson, S. N., and U. N. Nielsen. 2012. Foraging in the dark - Chemically mediated host plant location by belowground insect herbivores. J. Chem. Ecol. 38: 604–614.

Kaplan, F., H. T. Alborn, S. H. von Reuss, R. Ajredini, et al. 2012. Interspecific nematode signals regulate dispersal behavior. PLoS One 7: e38735. doi:10.1371/journal.pone.0038735.

Köllner, T. G., M. Held, C. Lenk, I. Hiltpold, et al. 2008. A maize (E)-β-caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. Plant Cell 20: 482–494.

Kurzt, B., I. Hiltpold, T. C. J. Turlings, U. Kuhlmann, et al. 2009. Comparative susceptibility of larval instars and pupae of the western corn rootworm to infection by three entomopathogenic nematodes. Biocontrol 54: 255–262.

Masamune, T., M. Anetai, M. Takasugi, and N. Katsui. 1982. Isolation of a natural hatching stimulus, glycinoeclepin-A, for the soybean cyst nematode. Nature 297: 495–496.

Mathesius, U., S. Mulders, M. S. Gao, M. Teplitski, et al. 2003. Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. P. Natl. Acad. Sci. USA. 100: 1444–1449.

Mitchell, P. 2011. Costs and benefits of controlling pest Diabrotica in maize in the United States, 24th IWGO Conference, Freiburg, Germany.

Mulder, J. G., P. Diepenhorst, P. Plieger, and I. E. M. Bruggemann-Rotgans inventors; B.V. Chemische Pharmaceutische Industrie "Luxan", PA Elst, Netherlands, assignee. 1992. Hatching agent for the potato cyst nematode. The Netherlands patent.

Rasmann, S., and A. A. Agrawal. 2008. In defense of roots: A research agenda for studying plant resistance to belowground herbivory. Plant Physiol. 146: 875–880.

Rasmann, S., A. C. Erwin, R. Halitschke, and A. A. Agrawal. 2011. Direct and indirect root defences of milkweed (*Asclepias syriaca*): trophic cascades, trade-offs and novel methods for studying subterranean herbivory. J. Ecol. 99: 16-25.

Rasmann, S., T. G. Köllner, J. Degenhardt, I. Hiltpold, et al. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434: 732–737.

Rasmann, S., I. Hiltpold, and J. G. Ali. 2012. The role of root-produced volatile secondary metabolites in mediating soil interactions. In Advances in selected plant physiology aspects, G. Montanaro and B. Dichio (eds.), ISBN: 9535105572 9789535105572, InTech, Rijeka, Croatia, 269–290.

Robert, C. A. M., M. Erb, M. Duployer, C. Zwahlen, et al. 2012. Herbivore-induced plant volatiles mediate host selection by a root herbivore. New Phytol. 194:1061–1069.

Schenk, H., R. A. J. Driessen, R. de Gelder, K. Goubitz, et al. 1999. Elucidation of the structure of Solanoeclepin A, a natural hatching factor of potato and tomato cyst nematodes, by single-crystal x-ray diffraction. Croat. Chem. Acta 72: 593–606.

Tanino, K., M. Takahashi, Y. Tomata, H. Tokura, et al. 2011. Total synthesis of solanoeclepin A. Nature Chem. 3: 484–488.

Toepfer, S., T. Haye, M. Erlandson, M. Goettel, et al. 2009. A review of the natural enemies of beetles in the subtribe *Diabroticina* (Coleoptera: Chrysomelidae): implications for sustainable pest management. Biocontrol Sci. Technol. 19: 1–65.

Turlings, T. C. J., I. Hiltpold, and S. Rasmann. 2012. The importance of root-produced volatiles as foraging cues for entomopathogenic nematodes. Plant and Soil 359: 51–60.

van Tol, R. W. H. M., A. T. C. van der Sommen, M. I. C. Boff, J. van Bezooijen, et al. 2001. Plants protect their roots by alerting the enemies of grubs. Ecol. Lett. 4: 292–294.

Wenke, K., M. Kai, and B. Piechulla. 2010. Belowground volatiles facilitate interactions between plant roots and soil organisms. Planta 231: 499–506.

Wesseler, J., and E. H. Fall. 2010. Potential damage costs of *Diabrotica virgifera virgifera* infestation in Europe - the 'no control' scenario. J. Appl. Entomol. 134: 385–394.

# Omnivory in spiders and the potential role of spider chemoreception in conservation biological control

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Spiders have historically been considered obligate predators and are known to be important predators of crop pests (Breene et al. 1989; Nyffeler et al. 1990; Ruberson and Greenstone 1986; Amalin et al. 2001; Miliczky and Calkins 2002; Richman 2003; Pfannenstiel 2005, 2008a). However, a growing body of evidence strongly suggests that omnivory plays an important role in the life history of spiders and that consumption of non-prey food resources, such as nectar, honeydew, pollen, and yeasts, can contribute materially to their survival, development, and reproduction. For example, high percentages of spiders collected in the field were positive for nectar-derived sugars (Taylor and Pfannenstiel 20089; Chen et al. 2010) demonstrating widespread nectar feeding in these spider populations. Nectar and honeydew sugars contributed to spider survival (Jackson et al 2001; Taylor and Bradley 2009; Pfannenstiel and Patt 2012) as well as to development and reproduction when the spiders were maintained on reduced prey diets (Taylor and Pfannenstiel 2009; Patt et al. 2012). Ingestion of pollen contributed directly to survival and, depending on the type of pollen, to development as well, both in the presence or absence of prey (Smith and Mommsen 1984; Vogelei and Greissl 1989; Pfannenstiel 2012; RS Pfannenstiel unpublished data). A few spider species have even been reared to adulthood on a diet comprised of pollen alone, and survival or developmental benefits of pollenivory have been observed in at least 12 spider families (Ludy 2004; Peterson et al. 2010; RS Pfannenstiel unpublished data). Combinations of pollen and honeydew have been observed to interact synergistically in support of spider development (RS Pfannenstiel unpublished data). Patt et al. (2012) recently demonstrated that commercial yeasts and soy powder could support spider development to adulthood under conditions of prey scarcity.

While searching for food, spiders respond similarly to prey and non-prey food resources and use contact chemoreception, olfaction, and restricted area search to assist them in locating food. Studies of spider searching behavior have shown that they use contact chemoreception (Persons and Uetz 1996; Punzo and Kukoyi 1997; Hoefler et al. 2002; Gallagher et al. 2012), as well as olfaction (Cross and Jackson 2009) to detect chemical cues associated with their prey. Patt and Pfannenstiel (2008) demonstrated that spiders can also use olfaction to detect nectar odors. Cursorial spiders engaged in restricted area search after contact with a single moth egg or tiny nectar droplet (Patt and Pfannenstiel 2009). Because both prey and non-prey food stimuli elicited the same behavioral response, it is likely that the presence of both types of food resources could contribute to spider retention in resource-rich habitats. Cursorial spiders can also learn to recognize novel odors and associate them with the presence of artificial nectar (Patt and Pfannenstiel 2008). This suggests that spiders use cognitive processes to locate and track resources that are temporally or spatially variable within their present environment.

The availability of non-prey food resources can benefit the plants providing them (Ruhren and Handel 1999; Whitney 2004; Romero et al. 2008). It is likely that there are many more cases where resource subsidies for spiders improve the biological control function performed by them. Omnivory in spiders appears to be common, and, as such, should be considered when strategies are developed to augment predation of pests using conservation biological control. Unlike many other predators (e.g. hemipterans, coccinellids) that actively immigrate into a crop system, spiders tend to be passive dispersers. Spider augmentation will be dependent on reducing secondary emigration after spiders initially enter a crop either from directly adjacent habitats or by ballooning. Behavioral responses, such as arrestment, localized searching behavior, associative learning, and chemotaxis, to the stimuli present in resource-augmented habitats are likely to result in reduced secondary emigration. Different types of resource augmentation strategies; i.e. interplanting with nectaried plants, application of food sprays, and banker plants, should be evaluated to determine their potential for retaining and supporting spider populations with the goal of improving biological control of crop pests.

## References

Amalin, D. M, J. Reiskind, J. E. Peña, and R. McSorley. 2001. Predatory behavior of three species of sac spider attacking citrus leafminer. J. Arach. 29: 72–81.

Breene, R. G., W. L. Sterling, and D. A. Dean. 1989. Predators of the cotton fleahopper on cotton. Southwest Entomol. 14: 159–166.

Chen, X., Y. Chen, L. Wu, Y. Peng, J. Chen, and F. Liu. 2010. A survey of nectar feeding by spiders in three different habitats. Bull. Insect. 63: 203–208.

Jackson, R., S. D. Pollard, X. J. Nelson, G. B. Edwards, and A.T. Barrion. 2001. Jumping spiders (Araneae: Salticidae) that feed on nectar. J. Zool. (Lond.) 255: 25–29.

Ludy, C. 2004. Intentional pollen feeding in the spider *Araneus diadematus* Clerck, 1757. Newsl. Br. Arach. Soc. 101: 4–5.

Lundgren, J. G. 2009. Relationships of Natural Enemies and Non-Prey Foods. Progress in Biological Control 7. Springer, USA.

Meissle, M., and J. Romeis. 2009. The web-building spider *Theridion impressum* (Araneae: Theridiidae) is not adversely affected by Bt maize resistant to corn rootworms. Plant Biotech. 7: 645–656.

Miliczky, E. R., and C. O. Calkins. 2002. Spiders (Araneae) as potential predators of leafroller larvae and egg masses (Lepidoptera: Tortricidae) in central Washington apple and pear orchards. Pan-Pacific Entomol. 78: 140–150.

Nyffeler, M., R. G. Breene, D. A. Dean, and W. L. Sterling. 1990. Spiders as predators of arthropod eggs. J. Appl. Entomol. 109: 490–501.

Patt J.M., R. S. Pfannenstiel, W. G. Meikle and J. J. Adamczyk. 2012. Supplemental diets containing yeast, sucrose, and soy powder enhance the survivorship, growth, and development of prey-limited cursorial spiders. Biol. Control. 63: 237–245.

Peterson J.A., S. A. Romero, and J. D. Harwood. 2010. Pollen interception by linyphiid spiders in a corn agroecosystem: implications for dietary diversification and risk-assessment. Arth.-Plant Int. 4: 207-217.

Pfannenstiel, R. S. 2005. Nocturnal predation and their impact on lepidopteran eggs in annual crops: What we don't see does help us!, pp. 463-471. *In* Proc. 2nd International Symposium on Biological Control of Arthropods. Davos, Switzerland, 12-16 Sept. 2005, USDA Forest Service, Morgantown, WV, FEHTET-2005-08, 734 p. 2005.

Pfannenstiel, R. S. 2008a. Spider predators of lepidopteran eggs in south Texas field crops. Biol. Contr. 46: 202–208.

Pfannenstiel, R.S. 2008b. Development of the cursorial spider, *Cheiracanthium inclusum* (Araneae: Miturgidae), on eggs of *Helicoverpa zea* (Lepidoptera: Noctuidae). J. Entomol. Sci. 43: 418–422.

Pfannenstiel, R. S. 2012. Direct Consumption of cotton pollen improves survival and development of *Cheiracanthium inclusum* (Araneae: Miturgidae) spiderlings. Ann. Entomol. Soc. Am. 105: 275–279.

Pfannenstiel, R. S., J. M. Patt, and F. Wäckers. 2008. Can extra-floral nectarines and other sugar based foods facilitate predation by cursorial spiders in row crop agroecosystems? pp. 277-284. *In* Proceedings 3rd International Symposium on Biological Control of Arthropods. P. G. Mason, D. R. Gillespie & C. Vincent. Christchurch, New Zealand, 8-13 Feb. 2009, USDA Forest Service, Morgantown, WV, FEHTET-2008-06, 636 p.

Pfannenstiel, R. S., and J. M. Patt. 2012. Feeding on nectar and honeydew sugars improves survivorship of two nocturnal cursorial spiders. Biol. Con. 63: 231-236.

Richman, D.B. 2003. The spiders (Araneae) of pecan groves in the southwestern United States and their role in pest suppression. Southwest Entomol. Suppl. 27: 115-122.

Romero, G. Q., J. C. Souza, and J. Vasconcellos-Neto. 2008. Anti-herbivore protection by mutualistic spiders and the role of plant glandular trichomes. Ecology 89: 3105-3115.

Ruberson, J.R., and M. H. Greenstone. 1998. Predators of budworm/bollworm eggs in cotton: an immunological study. pp. 1095-1098. *In* Proceedings, Beltwide Cotton Conference, National Cotton Council, Memphis, TN.

Ruhren, S., and S.N. Handel. 1999. Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. Oecologia 119: 227–230.

Smith, R. B., and T. P. Mommsen. 1984. Pollen feeding in an orb-weaving spider. Science. 226:1330-1332.

Taylor, R. M., and R. A. Bradley. 2009. Plant nectar increases survival, molting, and foraging in two foliage wandering spiders. J. Arach. 37: 232–237.

Taylor, R. M., and R. S. Pfannenstiel. 2009. How dietary plant nectar affects the survival, growth, and fecundity of a cursorial spider *Cheiracanthium inclusum* (Araneae: Miturgidae). Environ. Entomol. 38: 1379-1386.

Vogelei, A., and R. Greissl. 1989. Survival strategies of the crab spider *Thomisus onustus* Walckenaer 1806 (Chelicerata, Arachnida, Thomisidae). Oecologia 80: 513– 515.

Wäckers, F. L. 2005. Suitability of (extra-) floral nectar, pollen, and honeydew as insect food sources. pp. 17–74. *In* F.L. Wäckers, P. C. J. van Rijn, and J. Bruin (eds), Plant-provided Food for Carnivorous Insects.Cambridge University Press, Cambridge, UK.

Whitney, K. D. 2004. Experimental evidence that both parties benefit in a facultative plant-spider mutualism. Ecology, 85, 1642-1650.

# Session 11: Integrating Conservation Biological Control and Wildlife Conservation

## Beauty with benefits: naturescaping Washington vineyards to sustain biological control and provide butterfly habitat

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Arthropod pest management in Washington State wine grape, *Vitis vinifera* L. (Vitaceae), vineyards has recently transitioned from high to low-input and is now based on conservation biological control (CBC) and limited use of selective pesticides (Prischmann et al. 2005). Sustaining this biological control-based system will depend on the maintenance of robust and diverse populations of natural enemies. A promising strategy for increasing and sustaining predator and parasitoid populations within and adjacent to vineyards is the restoration of native plants and habitats, hosts and home to native natural enemies.

Wine grapes are primarily grown in the arid south central part of Washington which before irrigation was shrub-steppe dominated by sagebrush, *Artemisa* spp. (Asteraceae). The area of shrub-steppe has greatly diminished but patches exist adjacent to grape production areas. In addition to providing habitat and resources for endemic natural enemies, the shrub-steppe is also home to a diverse fauna of desert butterflies, most of which are declining, largely because of habitat destruction. Restoration of shrub-steppe habitat and native flora may potentially benefit butterfly and other pollinator populations as well as natural enemy populations of vineyard pests.

Beauty with Benefits is a research and extension program designed to enhance and sustain CBC in Washington wine grape vineyards while providing plant resources and habitat for native butterflies. Although our focus is on butterflies, restoration of shrub-steppe habitat will also benefit other native wildlife including reptiles, amphibians, birds and mammals.

Washington shrub-steppe is home to hundreds of species of flowering plants and bushes, all well-adapted to the hot, dry summers and cold winters that characterize the region. Unfortunately, there are no data on the relative attractiveness of different native plant species in Washington to beneficial insects. Thus, the first priority of our program was to obtain data on the incidence and abundance of predatory and parasitic insects attracted to different native flowering plants. In contrast, much is known about the larval host plants and nectar plants utilized by endemic butterfly species (James and Nunnallee 2011). Secondly, we compared 'native habitat-enhanced' to conventional vineyards in terms of pest, natural enemy and butterfly abundance.

### Methods

*Evaluation of beneficial insect attraction to native flowering plants.* Between 100 and 110 species of flowering shrubs, bushes and trees were evaluated in-situ using transparent sticky traps, for beneficial insect attraction. Insects were identified to species or family, counted and recorded. A replicated block experiment comparing beneficial insect attraction to 11 native plant species was also conducted.

*Evaluation of the pest, beneficial insect and butterfly faunas associated with habitat-enhanced and conventional vineyards.* Pests, beneficials and butterflies were monitored in eight pairs of commercial vineyards. Each pair comprised a 'native habitat-enhanced' (> 30 native plant spp.) and a conventional (< 7 native plant spp.) vineyard.

## Results

Attraction of beneficial insects to different native flowering plants varied substantially. A subset of ~40 flowering plants was identified, of species with apparent great attraction to one or more species or group of beneficials. Ten of these plants are shown in Table 1.

Table 1. Ten native plant species in south central Washington associated with high densities of beneficial insects and butterflies.

| Flowering Plant | Beneficials | Butterflies |
|---|---|---|
| *Eriogonum niveum* Douglas ex Benth. (Polygonaceae) | flies, parasitic wasps, bees | Lycaenidae (larval host)<br>nectar source |
| *Eriogonum compositum* Douglas ex Benth. (Polygonaceae) | ladybeetles, flies, para. wasps, predatory bugs | Lycaenidae (larval host)<br>nectar source |
| *Achillea millefolium* L. (Asteraceae) | predatory bugs, flies, parasitic wasps, bees | nectar source |
| *Asclepias speciosa* Torrey (Apocynaceae) | lacewings, flies, parasitic wasps, bees | *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae) (host), nectar source |
| *Eriophyllum lanatum* (Pursh) J. Forbes (Asteraceae) | flies, parasitic wasps | nectar source |
| *Clematis ligusticifolia* Nuttal (Ranunculaceae) | predatory bugs, flies, parasitic wasps | nectar source |
| *Ericameria nauseosa* (Pall. ex Pursh) G.L. Nesom & G.I. Baird (Asteraceae) | predatory bugs, parasitic wasps, bees | nectar source |
| *Lupinus* spp. (Fabaceae) | flies, parasitic wasps | Lycaenidae (larval host) |
| *Euthamia occidentalis* Nuttal (Asteraceae) | predatory bugs, parasitic wasps | nectar source |
| *Artemisia tridentata* Nuttal (Asteraceae) | predatory bugs, parasitic wasps, | |

Natural enemy populations were consistently greater and pest populations smaller in habitat-enhanced than in conventional vineyards. Butterfly species incidence and abundance were also greater in habitat-enhanced vineyards These preliminary data support the hypothesis that adjacent native habitats supporting a diverse community of native plants are an important source of predators, parasitoids and pollinators. In addition to providing direct benefits to viticulture, vineyard habitat restoration has the potential to help conserve native flora and fauna, including threatened butterfly species.

Identification of the most attractive flowering plants to specific beneficial insects (to combat specific grape pests) and butterflies will enable design and tailoring of native habitat in and around vineyards to provide effective CBC as well as provide refugia for desert butterflies threatened by diminishing habitats

## References

Prischmann, D. A., D. G. James, S. N. Gringas, and W. E. Snyder. 2005. Diversity and abundance of insects and spiders on managed and unmanaged grapevines in south central Washington State. Pan. Pac. Entomol. 81: 131–144.

James, D. G., and D. N. Nunnallee. 2011. Life Histories of Cascadia Butterflies. Oregon State University Press, Corvallis, Oregon, USA, 447 pp.

# Vertebrate conservation as an added ecosystem service from conservation biological control of pests

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Conservation biological control (CBC) of insects is the fastest-growing sub-discipline of biological control (Gurr et al. 2012, Wratten et al*.* 2013). This ecologically-founded approach involves an understanding of the ecological requirements of pests' natural enemies followed by "ecosystem engineering" (Gurr *et al.* 2004) to enhance key elements of the enemies' ecology. For example, using "soft" insecticides with reduced effects on non-target arthropods is included in CBC, as is reduced-rate application of insecticides as well as targeting their application in space and time, again to minimize effects on natural enemies. More usual, however, is the recognition of **SNAP** and its deployment in agro-ecosystems. SNAP summarises four main ways in which resources for natural enemies can be enhanced in agriculture: **S**helter, **N**ectar, **Al**ternative prey/hosts and **P**ollen. If farmers, farm advisors, researchers and students recognize the role that SNAP can play in CBC then this acronym can represent the beginnings of a more sustainable approach to pest control.

Biological control is an ecosystem service (ES), derived from predation and parasitism which are ecosystem functions (EF). ES represent ecosystem functions to which value to mankind, usually but not always financial, are ascribed i.e.

**EF + value to mankind = ES**

However, it would be naïve to expect that manipulating a crop environment in the above ways enhances only one ES. For example, in a well-known vinecology programme in New Zealand, deploying native and non-native plants to provide SNAP improves other ES, including soil quality and moisture and weed suppression (http://bioprotection.org.nz/greening-waipara).

Similarly, when floral resources are added to farmland to improve the fitness and pollination rates of bees and other insects, a suite of other ES improvements can be associated with that action (Wratten et al. 2012). Also, perhaps surprisingly, behavior and populations of vertebrates can also be enhanced when the initial target is a different ES. Key examples of this come from the iconic work on beetle banks in the UK, carried out by a team of researchers including Matthew Thomas, Nick Sotherton and Steve Wratten (Thomas et al. 1992, MacLeod et al*.* 2004). Although these banks were designed to enhance abiotic factors within farmland refugia for aphid predators such as carabid and staphylinid beetles, subsequent colonization processes of these banks led to high breeding populations of a rare mammal, the harvest mouse, *Micromys minutus* (Pallas) (Rodentia: Muridae), and the grey partridge, *Perdix perdix* (L.) (Galliformes: Phasianidae ) (Bence et al*.* 2003, Thomas et al*.* 2001). Densities of these two vertebrates can be higher on beetle banks and in fields containing this semi-natural cover than in any other UK farmland areas, largely because of the long stems of the dominant grass on beetle banks, cock's-foot (prairie grass), *Dactylis glomerata* L. (Poaceae). Densities of chick-food insects were as high on beetle banks as in existing field margins surrounding these cereal fields (Thomas et al*.* 2001). Chick-food insects are preferred as food items by the young of many farmland birds. These prey items include many phytophagous species but also a wide range of diurnal predatory beetles and spiders. The lack of sufficient insects during the chick rearing period is the key factor in the declines of many specialist farmland birds, especially the grey partridge (Potts 1986). In contrast, the agronomy of European cereals is such that the timing of machine harvesting destroys the nests of harvest mice while field boundaries with substantial cocksfoot populations, ideal habitats for grey partridge nesting, have declined in quantity and quality largely through hedgerow removal and the action of fertilizers and herbicides entering the base of the hedgerow (Rew et al. 1992, Boatman et al. 1994, Longley et al. 1997). Beetle banks can replace this nesting cover for ground-nesting species such as grey partridge (Thomas 2000). This paper will review progress and prospects in this area of multiple-ES provision.

## References

Bence, S. L., K. Stander, and M. Griffiths. 2003. Habitat characteristics of harvest mouse nests on arable farmland. Agric. Ecosyst. Environ. 99: 179–186.

Boatman, N. D., L. J. Rew, A. J. Theaker and R. J. Froud-Williams. 1994. The impact of nitrogen fertilizers on field margin flora. BCPC Monograph No 58: Field Margins: Integrating Agriculture and Conservation, pp. 209–214.

Longley, M., T. Cilgi, P.C. Jepson, and N.W. Sotherton. 1997. Measurements of pesticide spray drift deposition into field boundaries and hedgerows: 1. Summer applications. Environ. Toxicol. Chem. 16: 165–172.

Gurr, G., S. D. Wratten, and M. Altieri (eds.). 2004. Ecological Engineering for Pest Management: Advances in Habitat Manipulation for Arthropods. CSIRO Publishing, Melbourne, 256 pp.

Gurr, G. M., S. D. Wratten, and W. E. Snyder (eds.). 2012. Biodiversity and Insect Pests: Key issues for sustainable management. Wiley Blackwell Publishers, Hoboken, NJ, USA.

Potts, G. R. 1986. The Partridge. Collins, London, UK.

Rew, L. J., A. J. Theaker and R. J. Froud-Williams. 1992. Nitrogen fertilizer misplacement and field boundaries. Ann. Appl. Biol. 30: 203–206.

Thomas, S. 2000. Progress on beetle banks in UK arable farming. Pesticide Outlook 11: 51–53.

Thomas, S. R., D. Goulson, and J. M. Holland. 2001. Resource provision for farmland gamebirds: the value of beetle banks. Ann. Appl. Biol. 139**:** 111–118.

Wratten, S. D., H. Sandhu, R. Cullen, and R. Costanza (eds.) (2013) Ecosystem Services in Agricultural and Urban Landscapes. Wiley Blackwell Publishers, Hoboekn, NJ, USA (in press).

Wratten, S.D., M. Gillespie, A. Decourtye, E. Mader, and N. Desneux. 2012. Pollinator habitat enhancement: benefits to other ecosystem services. Agric. Ecosyst. Environ. 159: 112–122.

# The implications of biodiversity loss for pest population regulation: a multi-trophic perspective

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Biodiversity loss, driven by agricultural intensification and landscape homogenisation is known to occur across multiple trophic levels (Attwood et al. 2008, Flynn et al. 2009). However, the vast majority of studies into the functional implications of biodiversity loss have manipulated diversity at single trophic levels. This may be problematic for the prediction of biological control responses to biodiversity loss because there is much evidence to suggest that the functional impact of natural enemy species loss may be affected by interactions between trophic levels. For example, it has been predicted that the decline of function associated with predator species loss will depend on whether prey species diversity also declines (Worm and Duffy 2003). We also know that the impact of predator species loss on pest population suppression is likely to vary, depending on the development mode of the pest species concerned (Wilby and Thomas 2002a, Wilby et al. 2005). We can predict, therefore, that the effects of predator species loss are likely to be mitigated by parallel changes in the community and species attributes at the prey trophic level.

Prey attributes influence the functional responses to predator assemblages to diversity loss because the mechanisms underlying diversity effects in predator assemblages are dependent on the ecological context, in addition to the traits of the predators themselves. For example, ecologically differentiated life stages within a prey species (Wilby and Thomas 2002a, b) or differentiated niches among prey species are predicted to promote niche differentiation among predators, and possibly reduce intra-guild predation, leading to positive predator diversity effects on pest suppression (Wilby and Thomas 2007, Tylianakis and Romo 2010). The other principal mechanism underlying positive predator diversity effects occurs when there is facilitation among predator species (Losey and Denno 1998), which is often mediated by prey behaviour and is consequently likely to be prey species-specific. Predicting the net outcome of concurrent biodiversity change at multiple trophic levels is, therefore, far from simple.

Here we report a series of experiments designed to test whether interactions between predator species richness and prey species and/or community attributes influence predator biomass accumulation and the strength of pest suppression. Using a pool of four aphid species, *Aphis fabae* Scopoli and *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) feeding on bean, *Phaseolus vulgaris* L. (Fabaceae), and *Rhopalosiphum padi* (L.) and *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae) feeding on wheat, *Tritium aestivum* L. (Poaceae) and three species of aphidophagous larvae, *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae) we constructed model communities in which aphid species richness (one, two or four species) and predator species richness (one, two or three species) were independently manipulated in a total of 72 treatment combinations. The design also allowed us to test the effect of plant species richness by having aphid communities occupying a single or two plant species. Biomass change at all trophic levels was recorded, as was the population growth of each aphid species individually.

As predicted by biodiversity – ecosystem functioning theory, increased predator species richness was associated with higher biomass gain at the predator level, but this effect only occurred in treatments with a single herbivore species. As herbivore richness increased, biomass gain by the multi-predator assemblages fell below expectation, i.e. contrary to prediction, increased prey species richness negated positive predator richness effects on predator production. While all predator treatments had a strong suppressive effect on the aphid populations, increased aphid species richness resulted in increased total aphid biomass, particularly where aphid assemblages fed on two plant species rather than one. Statistical analysis revealed that in addition to a positive richness – the biomass production relationship among the aphid species, this pattern was also caused by decreased top-down control by predators as aphid richness increased. Further analysis of the population growth data of the individual aphids, supported by nitrogen isotope ratios of the predators, revealed that population growth of the wheat-feeding aphids was facilitated by the presence bean-feeding aphids, i.e. predator preferences for the bean-feeding aphids allowed compensatory population increases of the wheat-feeding aphids. It was also shown that positive predator diversity effects on prey suppression were restricted to low-productivity aphid treatments, supporting other studies which have shown that predator diversity effects may be density dependent (Griffiths et al*.* 2008).

In conclusion, our study showed that the impact of predator species loss on biological pest control may be strongly mediated by concurrent changes in the species diversity, composition, community structure and productivity of the prey community. Tentatively, we suggest that the functional benefits of predator richness may only occur in situations where the predator: prey ratio is relatively high, a scenario in which the benefit to biological pest control may be small. Our results also suggest that the positive predator richness effects may be more likely in relatively simple communities where pest diversity is very low, and less likely in intercrop situations, particularly if there are strong differences in prey quality between pest species. Although these conclusions are well supported by our data, we exercise caution in extending them to field situations as our results arise from mesocosm experiments at small temporal and spatial scales. We call for further study of the diversity effects in multi-trophic systems at larger scales and stress that as scales increase, other factors are likely to come into play that may result in a more detrimental impact of species loss on biological pest control (Loreau et al. 2003, Loreau 2004, Wilby and Thomas 2007, Tylianakis and Romo 2010).

## References

Attwood, S. J., M. Maron, A. P. N. House, and C. Zammit. 2008. Do arthropod assemblages display globally consistent responses to intensified agricultural land use and management? Global Ecol. Biogeogr. 17: 585–599.

Flynn, D. F. B., M. Gogol-Prokurat, T. Nogeire, N. Molinari, B. T. Richers, B. B. Lin, N. Simpson, M. M. Mayfield, and F. DeClerck. 2009. Loss of functional diversity under land use intensification across multiple taxa. Ecol. Lett. 12: 22–33.

Griffiths, G. J. K., A. Wilby, M. J. Crawley, and M. B. Thomas. 2008. Density-dependent effects of predator species-richness in diversity-function studies. Ecology 89: 2986–2993.

Loreau, M. 2004. Does functional redundancy exist? Oikos 104: 606–611.

Loreau, M., N. Mouquet, and A. Gonzales. 2003. Biodiversity as spatial insurance in heterogeneous landscapes. P. Natl. Acad. Sci. USA 100: 12765–12770.

Losey, J. E., and R. F. Denno. 1998. Positive predator-predator interactions: Enhanced predation rates and synergistic suppression of aphid populations. Ecology 79: 2143–2152.

Tylianakis, J. M., and C. M. Romo. 2010. Natural enemy diversity and biological control: Making sense of the context-dependency. Basic Appl. Ecol. 11: 657–668.

Wilby, A., and M. B. Thomas. 2002a. Natural enemy diversity and natural pest control: patterns of pest emergence with agricultural intensification. Ecol. Lett. 5: 353–360.

Wilby, A., and M. B. Thomas. 2002b. Are the ecological concepts of assembly and function of biodiversity useful frameworks for understanding natural pest control? Agric. Forest Entomol. 4: 237–243.

Wilby, A., and M. B. Thomas. 2007. Diversity and pest management in agro-ecosystems - some perspectives from ecology, pp. 225–269. In D. I. Jarvis, C. Padoch, and H. D. Cooper (eds.) Managing biodiversity in agricultural ecosystems. Columbia University Press, New York, NY, USA.

Wilby, A., S. Villareal, L. Lan, K. Heong, and M. Thomas. 2005. Functional benefits of predator species diversity depend on prey identity. Ecol. Entomol. 30: 497–501.

Worm, B., and J. E. Duffy. 2003. Biodiversity, productivity and stability in real food webs. T. Ecol. Evol. 18: 628–632.

# Milkweed: A resource for increasing stink bug parasitism and aiding insect pollinator and monarch butterfly conservation

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The flowers of milkweed, *Asclepias* spp. (Apocynaceae), can produce a rich supply of nectar, and therefore, planting an insecticide-free milkweed habitat in agricultural farmscapes could possibly conserve monarch butterflies, bees and other insect pollinators, as well as enhance parasitism of insect pests. In peanut-cotton farmscapes in Georgia, stink bugs, i.e. *Nezara viridula* (L.), *Euschistus servus* (Say), and *Chinavia hilaris* (Say), (Hemiptera: Pentatomidae) develop in peanut and then disperse at the crop-to-crop interface to feed on fruit in cotton. Strategic placement, in time and space, of a milkweed habitat at these crop-to-crop interfaces may lead to successfully increasing biological control of stink bugs in these agricultural farmscapes. Specifically, this work aimed to: 1) document feeding of the monarch butterfly, *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae), insect pollinators and stink bug parasitoids on milkweed nectar in the field, 2) determine the impact of strategic placement of milkweed nectar provision on parasitism rates of stink bugs in cotton, and 3) and examine the ability of the milkweed insectary habitat to reduce stink bugs below economic threshold in cotton. In 2009, plots of cotton (22.9 m long by 1.83 m [16 rows] deep) were established along the interface of a peanut-cotton farmscape. Each treatment, cotton with a milkweed insectary habitat and cotton control, was randomly assigned to a plot within a block for each of 4 blocks in a randomized complete block (RCB) design (2 treatments × 4 replicates). In 2010, there were 5 treatment replicates. For the insectary habitat, 25 potted flowering milkweed plants were placed 1.2 m apart along the edge of a cotton plot. Stink bugs were sampled each week for 5 weeks during stink bug colonization of cotton. For each sample, all plants within a 1.83-m length of row were shaken over a drop cloth and visually checked for these bugs. Over the 2-yr study, monarch butterflies, insect pollinators and stink bug parasitoids readily fed on milkweed nectar in these insecticide-free habitats. For the first year of the study, addition of the milkweed insectary habitat adjacent to cotton increased parasitism of *N. viridula* by *Trichopoda pennipes* (Fabricius) (Diptera: Tachinidae) by close to 5-fold relative to control cotton. In 2010, combined parasitism of *N. viridula*, *C. hilaris*, and a leaffooted bug, *Leptoglossus phyllopus* (L.) (Hemiptera: Coreidae), by *T. pennipes* was at least 3-times greater in plots with a milkweed habitat compared to control cotton. For each year of the study, differences in parasitism rates between treatments were not due to differences in bug density. In 2009, there was some indication that this sole management strategy can help maintain stink bugs below economic threshold in this crop. However, complementary management strategies, such as use of selective insecticides and trap cropping, may also need to be incorporated in these farmscapes to continually suppress these pests below economic threshold throughout the growing season. In conclusion, provision of a milkweed insectary habitat at the crop-to-crop interface in peanut-cotton farmscapes aided insect pollinator and monarch butterfly conservation as well as increased the parasitism rate of adult stink bugs in cotton.

# Session 12: Conservation Biological Control: A Landscape Perspective

## Influence of landscape heterogeneity on biological control of the western grape leafhopper (*Erythroneura elegantula* Osborn) in northern California vineyards

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Modern agriculture is characterized by monoculture, as growers attempt to maximize yield and profits by taking advantage of economies of scale and specialized production. Such a reduction in agro-biodiversity can result in habitat loss for natural enemies while simultaneously increasing the concentration of plant-host resources for potential arthropod pests. Together these two processes can disrupt biological control of pests and/or lead to pest outbreaks (Root 1973, Letourneau 1987, Landis et al. 2000). At present many farmers, including California grape growers, primarily rely on the use of chemical controls for pest management (Bentley 2009). Yet at present the continued availability of many chemical controls has come into question largely due to regulation of products following evidence of non-target impacts but also due to the development of pesticide resistance, rising application costs, as well as consumer demand for sustainable production practices (Whalon et al. 2008, Howard and Allen 2010).

In anticipation of such regulations, many California wine grape growers have demonstrated support for the development of ecologically-based pest management practices, in particular the use of on-farm habitat diversification (Wine Institute 2002, Ohmart 2011). Previous research has shown that on-farm (i.e., field scale) habitat diversification generally appears to support increased natural enemy populations and lower herbivore populations (Letourneau et al. 2011, Tonhasca and Byrne 1994, Andow 1991, Russel 1989). Similarly, in the absence of any field scale diversification, changes in habitat diversity at the landscape scale (i.e., >1 km. radius around crop fields) can itself influence natural enemy and pest populations (Bianchi et al. 2006, Gardiner et al. 2009). As such, it is thought that the effectiveness of on-farm habitat diversification to enhance biological control will likely be influenced by landscape heterogeneity (Tscharntke et al. 2007, Weibull et al. 2003). While this has recently been demonstrated in multiple cropping systems (Eilers and Klein 2009, Weibull et al. 2003, Ostman et al. 2001), the causal mechanisms are thought to be system specific.

Since the 1970s the California wine grape industry has experienced tremendous growth. In the popular north coast region (Napa, Sonoma, Mendocino and Lake County), the intensification and expansion of grape production over the past 40 years has led to an aggregate reduction in the area and quality of natural habitats and an agricultural landscape almost entirely dominated by wine grape vineyards (Merenlender 2000). To this point, more recent studies have shown that changes in the area and quality of natural habitats surrounding a vineyard can influence spider species composition within the vineyard (Hogg and Daane 2011a, 2011b, Isaia et al. 2006). In California, spiders are the dominant generalist predator in vineyards (Costello and Daane 1995) and the relationship between natural habitats surrounding vineyards and the diversity of spiders found within those vineyards could have important implications for biological control of grape pests.

In the vineyard agroecosystem, it is very likely that landscape heterogeneity will also influence the effectiveness of on-farm habitat diversification because it is known that the key egg-parasitoid, (*Anagrus* spp. ([Hymenoptera: Mymaridae]), of the grape leafhopper, (*Erythroneura elegantula* Osborne ([Homoptera: Cicadellidae]), must overwinter on alternate leafhopper host species found in habitats outside of vineyards. For example, California studies have shown that *Anagrus* spp. can overwinter on the blackberry leafhopper, (*Dikrella californica* (Lawson) [(Homoptera: Cicadellidae]), and prune leafhopper, (*Edwardsiana prunicola* (Edwards) [(Homoptera: Cicadellidae]), (Doutt and Nakata 1965, Kido et al. 1984) and similar work has been conducted in British Columbia (Lowery et al. 2007), Washington (Wright and James 2007), and New York (Williams and Martinson 2000). Previous vineyard studies have attempted to manipulate local habitat diversity to enhance populations of *Anagrus* spp. to increase egg parasitism of the grape leafhopper. In these studies increased local habitat diversity provided either overwintering habitat (Flaherty et al. 1985; Murphy et al. 1996, 1998) or seasonal resources for natural enemies (Nicholls et al. 2000, 2001; Daane and Costello 1998; Costello and Daane 2003; English-Loeb et al. 2003). While these studies demonstrated that habitat diversification can sometimes lead to a reduction in leafhopper densities, the ecological mechanisms are not entirely clear. Subsequent work has elucidated *Anagrus* movement (Corbett and Rosenheim 1996, Corbett et al. 1996), species identification (Triapitsyn 1998) and longevity benefits from nectar feeding (English-Loeb et al. 2003). In this context, the influence of landscape heterogeneity on *Anagrus* populations and biological control of leafhoppers has never been fully explored in vineyards nor has the interaction between local and landscape habitat diversity. The inconsistent results from previous diversification trials could potentially be due to the influence of landscape heterogeneity on the effectiveness of such practices and, as such, this project is the first ever to evaluate this hypothesis in vineyards.

The results presented here are part of a larger, multi-year collaboration between researchers at the University of California - Berkeley and more than 25 commercial wine grape growers. The goal of this collaborative effort is to develop and evaluate ecologically-based pest management practices for wine grape vineyards. The objective of the current research is to understand how pests and their natural enemies utilize non-crop habitats surrounding vineyards and whether this subsequently influences biological control in monocultures as well as to evaluate the effectiveness of on-farm floral resource provisioning to enhance biological control in a variety of landscapes.

Presented here are the preliminary results from a 3-year study that focused on vineyard monocultures that were spread along a continuum of landscape heterogeneity. All vineyard sites were located in Napa and Sonoma County, California, and consisted of red varietals (merlot, cabernet sauvignon etc.) that were >5-years old. All plots were kept insecticide free, with the exception of mandatory sprays for the European grapevine berry-moth, (*Lobesia botrana* (Denis & Schiffermüller ([Lepidoptera:Tortricidae]), a recently invasive pest targeted for eradication (Varela et al. 2010). All pesticides used for control of *L. botrana* at research sites were indicated as having no/low toxicity to natural enemies and leafhopper pests (Cooper 2010).

During the 2010-2012 growing seasons, data was collected on natural enemy and pest populations, pest parasitism rates and vine vigor at 20 vineyard sites. Landscape heterogeneity was then quantified at 7 spatial scales around each site (radii of 0.5 – 6 km). Insect data was assessed at each spatial scale using multiple regression in order to evaluate whether changes in the area and quality of natural habitats influence insect populations and biological control of *E. elegantula*. The relationship between vine vigor and *E. elegantula* populations will also be evaluated, as previous studies have demonstrated leafhopper preference for more vigorous vines (Daane and Williams 2003, Daane and Costello 1998). A natural enemy exclusion study was also conducted in a subset of these vineyard sites to evaluate whether natural enemy impact varied with changes in landscape heterogeneity.

## References

Andow, D. A. 1991. Yield loss to arthropods in vegetationally diverse agroecosystems. Environ. Entomol. 20: 1228–1235.

Bentley, W. 2009. The integrated control concept and its relevance to current integrated pest management in California fresh market grapes. Pest Manage. Sci. 65: 1298–1304.

Bianchi, F., C. J. H. Booij, and T. Tscharntke. 2006. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. Proc. R. Soc. B. 273: 1715–1727.

Cooper, M. L. 2010. Insecticides for *Lobesia botrana* on grape. Bull. – Univ. Calif., Div. Agric. Sci.

Corbett, A., and J. A. Rosenheim. 1996. Impact of a natural enemy overwintering refuge and its interaction with the surrounding landscape. Ecol. Entomol. 21: 155–164.

Corbett, A., B. C. Murphy, J. A. Rosenheim, and P. Bruins. 1996. Labeling an egg parasitoid, *Anagrus epos* (Hymenoptera: Mymaridae), with rubidium within an overwintering refuge. Environ. Entomol. 25: 29-38.

Costello, M. J., and K. M. Daane. 1995. Spider (Araneae) species composition and seasonal abundance in San Joaquin Valley grape vineyards. Environ. Entomol. 24:823–831.

Costello, M. J., and K. M. Daane. 2003. Spider and leafhopper (*Erythroneura* spp.) response to vineyard ground cover. Environ. Entomol. 32: 1085–1098.

Daane, K. M., and M. Costello. 1998. Can cover crops reduce leafhopper abundance in vineyards? Calif. Agric. 52: 27–33.

Daane, K. M., and L. E. Williams. 2003. Manipulating vineyard irrigation amounts to reduce insect pest damage. Ecol. Appl. 13: 1650–1666.

Doutt, R. L., and J. Nakata. 1965. Overwintering refuge of *Anagrus epos* (Hymenoptera: Mymaridae). J. Econ. Entomol. 58: 586.

Doutt, R. L., and J. Nakata. 1973. The *Rubus* leafhopper and its egg parasitoid: an endemic biotic system useful in grape-pest management. Environ. Entomol. 2: 381–386.

Eilers, E. J., and A. M. Klein. 2009. Landscape context and management effects on an important insect pest and its natural enemies in almond. Biol. Control 51: 388–394.

English-Loeb, G., G. M. Rhainds, T. E. Martinson, and T. A. Ugine. 2003. Influence of flowering cover crops on *Anagrus* parasitoids (Hymenoptera: Mymaridae) and *Erythroneura* leafhoppers (Homoptera: Cicadellidae) in New York vineyards. Agric. For. Entomol. 5: 173–181.

Flaherty, D. L., L. T. Wilson, V. M. Stern, H. Kido. 1985. Biological control in San Joaquin Valley vineyards, pp. 501-520. *In* M. A. Hoy, and D. C. Herzog (eds.), Biological Control in Agricultural IPM Systems. Academic Press: New York, NY.

Gardiner, M. M., D. A. Landis, C. Gratton, C. D. DiFonzo, M. O'Neal, J. M. Chacon, M. T. Wayo, N. P. Schmidt, E. E. Mueller, and G. E. Heimpel. 2009. Landscape diversity enhances biological control of an invasive pest in the north-central USA. Ecol. Appl. 19: 143–154.

Hogg, B. N., and K. M. Daane. 2011a. Ecosystem services in the face of invasion: the persistence of native and nonnative spiders in an agricultural landscape. Ecol. Appl. 21: 565–576.

Hogg, B. N., and K. M. Daane. 2011b. Impacts of a dominant non-native predator on predator diversity and herbivore suppression. J. Appl. Ecol. 48: 453-461.

Howard, P. H., and P. Allen. 2010. Beyond organic and fair trade?: an analysis of ecolabel preferences in the United States. Rural Sociol. 75: 244–269.

Isaia, M., F. Bona, and G. Badino. 2006. Influence of landscape diversity and agricultural practices on spider assemblage in Italian vineyards of Langa Astigiana (Northwest Italy). Environ. Entomol. 35: 297–307.

Kido, H., D. Flaherty, D. Bosch, and K. Valero. 1984. French prune trees as overwintering sites for the grape leafhopper egg parasite. Am. J. Enol. Vitic. 35: 156.

Landis, D. A., S. D. Wratten, and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45: 175–201.

Letourneau, D. K. 1987. The enemies hypothesis: tritrophic interactions and vegetational diversity in tropical agroecosystems. Ecology 68:1616–1622.

Letourneau, D. K., I. Armbrecht, B. S. Rivera, J. M. Lerma, E.J . Carmona, M.C . Daza, S. Escobar, V. Galindo, C. Gutiérrez, S. D. López, J. L. Mejía, A. M. A. Rangel, J. H. Rangel, L. Rivera, C. A. Saavedra, A. M. Torres, and A. R. Trujillo. 2011. Does plant diversity benefit agroecosystems? a synthetic review. Ecol. Appl. 21:9–21.

Lowery, D. T., S. V. Triapitsyn, and G. J. R. Judd. 2007. Leafhopper host plant associations for *Anagrus* parasitoids (Hymenoptera: Mymaridae) in the Okanagan Valley, British Columbia. J. Entomol. Soc. B. C. 104: 9–16.

Merenelender, A. 2000. Mapping vineyard expansion provides information on agriculture and the environment. Calif. Agric. May-June 2000.

Murphy, B. C., J. A. Rosenheim, and J. Granett. 1996. Habitat diversification for improving biological control: abundance of *Anagrus epos* (Hymenoptera: Mymaridae) in grape vineyards. Environ. Entomol. 25: 495–504.

Murphy, B. C., J. A. Rosenheim, R. V. Dowell, and J. Granett. 1998. Habitat diversification tactic for improving biological control: parasitism of the western grape leafhopper. Entomol. Exp. Appl. 87: 225–235.

Nicholls, C. I., M. P. Parrella, and M. A. Altieri. 2000. Reducing the abundance of leafhoppers and thrips in a northern California organic vineyard through maintenance of full season floral diversity with summer cover crops. Agric. For. Entomol. 2: 107–113.

Nicholls, C. I., M. Parrella, and M. A. Altieri. 2001. The effects of a vegetational corridor on the abundance and dispersal of insect biodiversity within a northern California organic vineyard. Landsc. Ecol. 16: 133–146.

Ohmart, C. O. 2011. View from the vineyard: a practical guide to sustainable winegrape growing. Wine Appreciation Guild of San Francisco, San Francisco, CA.

Ostman, O., B. Ekbom, and J. Bengtsson. 2001. Landscape heterogeneity and farming practice influence biological control. Basic Appl. Ecol. 2: 365-371

Root, R. B. 1973. Organization of plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*) Ecol. Monogr. 43: 95–124.

Russell, E. P. 1989. Enemies hypothesis: a review of the effect of vegetational diversity on predatory insects and parasitoids. Environ. Entomol. 18: 590–599

Tonhasca, A., and D. N. Byrne. 1994. The effects of crop diversification on herbivorous insects: a meta-analysis approach. Ecol. Entomol. 19: 239–244.

Triapitsyn, S. V. 1998. *Anagrus* (Hymenoptera: Mymaridae) egg parasitoids of *Erythroneura* spp. and other leafhoppers (Homoptera: Cicadellidae) in North American vineyards and orchards: a taxonomic review. Trans. Amer. Entomol. Soc. 124: 77–112.

Tscharntke, T., I. Steffan-Dewenter, A. Kruess, and C. Thies. 2002. Contribution of small habitat fragments to conservation of insect communities of grassland-cropland landscapes. Ecol. Appl. 12: 354–363.

Tscharntke, T., R. Bommarco, Y. Clough, T. O. Crist, D. Kleijn, T. A. Rand, J. M. Tylianakis, S. van Nouhuys, and S. Vidal. 2007. Conservation biological control and enemy diversity on a landscape scale. Biol. Control 43: 294–309.

Varela, L. G., R. J. Smith, M. L. Cooper, and R. W. Hoenisch. 2010. European grapevine moth, *Lobesia botrana*, in Napa valley vineyards. Pr. Winer. March/April 2010.

Weibull, A. C., O. Ostman, and A. Granqvist. 2003. Species richness in agroecosystems: the effect of landscape, habitat and farm management. Biodivers. Conserv. 12: 1335-1355.

Whalon, M. E., D. Mota-Sanchez, and R. M. Holingworth (eds.). 2008**.** Global pesticide resistance in arthropods. Cab International, Cambridge, MA.

Williams, L., and T. Martinson. 2000. Colonization of New York vineyards by *Anagrus* spp. (Hymenoptera: Mymaridae): overwintering biology, within-vineyard distribution of wasps, and parasitism of grape leafhopper, *Erythroneura* spp. (Homoptera: Cicadellidae), eggs. Biol. Control 18: 136–146.

(WI) Wine Institute. 2010. Highlight of the month: sustainable pest management. Wine Institute, San Francisco, CA.

Wright, L. C., and D. G. James. 2007. *Anagrus* spp. (Hymenoptera: Mymaridae) reared from plants collected during winter in south central Washington and north central Oregon. J. Entomol.Soc. B. C. 104: 17–24.

# Spatial organization of organic and conventional farming in agricultural landscapes: impacts on beneficial insects

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Given the current decline of biodiversity, modern agriculture is facing a new challenge: ensuring food supply to an increasingly large human population whilst maintaining the ecological balance of agro-ecosystems to preserve their sustainability. Because of a strong reduction of chemical inputs, organic farming is considered as a promising solution to promote communities of natural enemies and ensure crop protection thanks to biological control. Indeed, this ecological service seems to be an interesting alternative to chemical control but its functioning is not well understood.

Numerous studies have investigated the effect of organic *vs*. conventional farming on biodiversity at the field or farm scale. Overall, they show a positive effect of organic practices on the diversity or abundance of natural enemies, but there are also contradictory results (Bengtsson *et al.* 2005). This might be explained by the dichotomy generally made between organic and conventional farming, which does not always make sense because of the large diversity of practices occurring in each farming type (Laurens unpublished). The effect of organic farming on natural enemies and biological control was also shown to vary according to landscape heterogeneity (Burel and Baudry 2003) or complexity (usually described by the amount of semi-natural elements) (Rundlöf & Smith 2006, Holzschuh *et al.* 2010), which is recognized as a key driver of biodiversity. Furthermore, recent studies suggest that biodiversity is enhanced in landscapes with large areas of organic farming (Gabriel *et al.* 2010). However, the effects of the diversity and spatial organization of organic and conventional farming practices remain unexplored.

The present study aims at measuring the influence of the diversity and spatial organization of organic and conventional farming practices on aphids and their natural enemies (ladybirds, predatory carabid beetles and parasitoids) in winter wheat. Higher abundances of beneficial insects were expected in organic fields and “organic landscapes” but with important variations due to practices diversity. Moreover, fields directly surrounded by extensive practices, either organic or conventional, were expected to present high abundance of natural enemies.

Twenty, 1 km² landscapes were selected in Brittany (Western France). These landscapes were characterized by similar coverage of semi-natural elements, farmland, built areas and water, but different amount of organic fields (from 6 to 37%). In each landscape, ladybirds, carabid beetles, parasitoids and aphids communities were sampled from April to July 2012 in one pair of organic and conventional winter wheat fields. In each of the 40 fields, data on vegetation height, density, and weeds cover were also collected as descriptors of abiotic conditions. Interviews of farmers were conducted to describe agricultural practices (crop management sequence, seeding, winter cover, previous crop and crop rotation) on sampled and surrounding fields.

The results presented here concern ladybirds and include data on diversity of farming practices at the field scale only. At the field scale, the analysis of agricultural practices on wheat fields was done using Correspondence Analysis (Teil, 1975). Fields coordinates along the first three CA axes were used in linear models as descriptors of farming practices. Only field coordinates along the first axis had an effect on ladybirds. This axis was mostly related to farming type, with a distinction of organic (no inputs, frequent tillage and organic fertilization, long rotations) *vs.* conventional practices (chemical inputs, low tillage and organic fertilization, short rotations). However, there was a diversity of practices among interviewed farmers of each farming type (Figure

1a). Ladybirds were more abundant in fields with practices close to organic farming. This is consistent with studies which found direct or indirect lethal effects of chemical pesticides on ladybirds (Obrycki & Kring 1998). On the contrary, ladybirds are positively affected by frequent tillage and organic fertilization. Tillage is unlikely to affect ladybirds as they are absent in fields when most interventions are done (autumn), but some studies demonstrate a positive effect of organic fertilization on natural enemies (Holland & Luff 2000, Garratt *et al.* 2011). In addition, organic fields were characterized by dense weed cover compared to conventional fields. It might result from the practices previously mentioned, and explain their positive effect on ladybirds. Indeed, weeds create favorable and attractive microclimatic conditions thanks to vegetation structure (Ali & Reagan 1985). No effect of aphids abundance was found, probably because there were not enough individuals this year.

At the landscape scale, the proportion of the boundary of studied fields with organic fields had a strong positive effect on the abundance of ladybirds (Figure 1b). At larger scale, ladybirds were positively impacted by the amount of organic farming in 250m buffers (Figure 1c). This is consistent with Gabriel *et al.* (2010), which demonstrate a positive effect of organic farming in 10 km² landscapes. This suggests that farming practices may impact insects beyond the field scale. In fact, it is shown that pesticides can widely disperse in the environment (Wittich & Siebers 2002). Furthermore, ladybirds are known to move frequently between habitats (Evans 2003) and can consequently be affected by practices at landscape scale. However, ladybirds were not influenced by landscape characteristics in 500m buffers, probably because the coverage of organic farming amongst sites varies less than in 250m buffers.

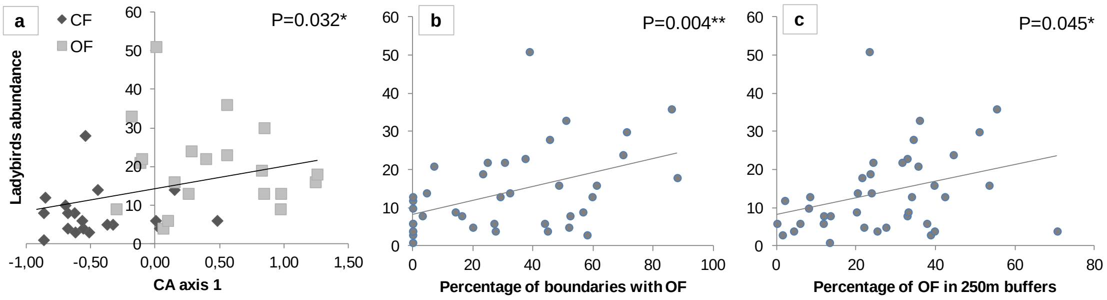

**Figure 1.** Effect on ladybirds abundance of a) the first CA axis (CF: Conventional Farming ; OF: Organic Farming) b) the percentage of boundaries between the study fields and organic fields c) the percentage of organic fields in 250m buffers. N=40.

To conclude, these results demonstrate that even if organic and conventional farming systems are basically different, there is a wide diversity of practices within each farming type that should be taken into account. Moreover, the strong effects of agricultural practices on ladybirds at field and landscape scales suggest that practices effects on natural enemies should be taken into account at different spatial scales to improve biological control efficiency.

Further analysis will be done to evaluate the effect of the detailed agricultural practices at landscape scale on ladybirds. Comparison with results for parasitoids and ground beetles will also provide additional information about the biological control potential.

## References

Ali, A. D., and T. E. Reagan. 1985. Vegetation manipulation impact on predator and prey populations in Louisiana sugarcane ecosystems. J. Econ. Entomol. 78: 1409–1414.

Bengtsson, J., J. Ahnström, and A.-C. Weibull. 2005. The effects of organic agriculture on biodiversity and abundance: a meta-analysis. J. Appl. Ecol. 42: 261–269.
Burel, F., and J. Baudry. 2003. Landscape Ecology: Concepts, methods and applications. Science Publishers, Enfield, NH, USA
Evans, E. W. 2003. Searching and reproductive behaviour of female aphidophagous ladybirds (Coleoptera: Coccinellidae): a review. Eur. J. Entomol. 100: 1–10.
Gabriel, D., S. M. Sait, J. A. Hodgson, U. Schmutz, W. E. Kunin, and T. G. Benton. 2010. Scale matters: the impact of organic farming on biodiversity at different spatial scales. Ecol. Lett. 13: 858–869.
Garratt, M. P. D., D. J. Wright, and S. R. Leather. 2011. The effects of farming system and fertilisers on pests and natural enemies: A synthesis of current research. Agr. Ecosyst. Environ. 141: 261–270.
Holland, J. M., and M. L. Luff. 2000. The effects of agricultural practices on Carabidae in temperate agroecosystems. Integrated Pest Management Reviews 5: 109–129.
Holzschuh, A., I. Steffan-Dewenter, and T. Tscharntke. 2010. How do landscape composition and configuration, organic farming and fallow strips affect the diversity of bees, wasps and their parasitoids? J. Anim. Ecol. 79: 491–500.
Laurens, E. 2012. Etude des pratiques en blé dans les exploitations bretonnes. Unpublished.
Obrycki, J. J., and T. J. Kring. 1998. Predaceous Coccinellidae in biological control. Annu. Rev. Entomol. 43: 295–321.
Rundlöf, M., and H. G. Smith. 2006. The effect of organic farming on butterfly diversity depends on landscape context. J. Appl. Ecol. 43: 1121–1127.
Teil, H. 1975. Correspondence factor analysis: An outline of its method. J. Int. Ass. Math. Geol. 7: 3–12.
Wittich, K. P., and J. Siebers. 2002. Aerial short-range dispersion of volatilized pesticides from an area source. Int. J. Biometeorol. 46: 126–135.

# Predicting biological control of cereal aphids across agricultural landscapes

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## Introduction

It has repeatedly been shown that complex landscapes host a higher abundance and diversity of natural enemies compared to simplified landscapes dominated by intensive agriculture (Bianchi et al. 2006, Chaplin Kramer et al. 2011). However, few studies have assessed how this affects the level and stability of biological control services across landscapes, and ecological models that can predict such relationships are lacking. Aphids are major pests on cereal crops such as wheat and barley in Europe (Östman et al. 2003), and both generalist predators such as spiders and ground beetles and specialist enemies like parasitoids, hoverflies and ladybeetles are known to be important biological control agents in this system (Wiktelius 1987, Schmidt et al. 2003).

First of all we present a field experiment where we estimated how the level and spatial stability of biological control of cereal aphids varied with landscape complexity and crop rotation intensity using predator exclusion cages (Rusch et al. in press). To provide a more mechanistic understanding of how different natural enemy taxa contribute to biological control of cereal aphids in different landscapes we have constructed a mechanistic model based on aphid population dynamics, predation rates and abundances of different natural enemy taxa in different landscapes. We present this model and discuss its predictions, how well it fits with validation data and how it might be developed further (Jonsson et al. unpubl.).

## Materials and methods

***Field study.*** In this study, we combined a "mensurative experiment" with experimental manipulation (predator exclusion cages) to examine the relative effects of landscape complexity and crop rotation intensity on the level and the stability of biological control of cereal aphids in southern Sweden. We also investigated the spatial scale that best predicted the overall natural pest control. By using a design in which complexity and crop rotation intensity in the landscape were orthogonal factors, we were able to disentangle the relative effects of these variables on pest control services.

***Modelling study.*** Using a production function approach (Daily et al. 2009), we developed a mechanistic landscape model for biological control of cereal aphids, explicitly accounting for the influence of landscape composition on natural enemies varying in mobility. As an explicit example, we used this model to map biological control services across cereal fields in a central Swedish agricultural region varying in landscape complexity. To parameterize the model we used literature reviews and analyses of our own data. The model was validated with one independent dataset (Östman et al. 2001).

## Results and Discussion

In the field study we found that the average level of biological control increased with landscape complexity, but surprisingly, the spatial stability of biological control increased with increasing crop rotation intensity in the landscape. Multiple spatial scales analyses showed that the mean level of natural pest control was best predicted by landscape complexity at the 0.5 km and 1 km spatial scales. The stability in overall pest control decreased with proportion of ley at the 2.5 km and 3 km spatial scales. Our study disentangled the relative effects of landscape complexity and crop rotation intensity on the delivery of biological control. We show that combined management of semi-natural habitat and crop rotation can stabilize and enhance biological control in agricultural landscapes.

The model predicted that biological control would reduce crop damage by 45 – 70% and that the biological control effect would be higher in complex landscapes. The relative contribution of different predator taxa to biological control varied with landscape composition, but generalist predators collectively contributed with about 50% of the biological control effect in most landscapes. The model was able to predict a significant proportion of biological control variation in cereal fields from independent data (Östman et al. 2001). However, much variability remains to be explained and we recommend how the model could be improved by refining the mechanistic understanding of predator dynamics and accounting for aphid colonization. If this kind of ecological production function is combined with similar production functions for other services the combination may become a powerful tool in planning for sustainable agriculture.

## References

Bianchi, F.J. J. A., C. J. H. Booij, and T. Tscharntke. 2006. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. P. R. Soc. B Biological Sciences 273: 1715–1727.

Chaplin-Kramer, R., M. E. O'Rourke, E. J. Blitzer, and C. Kremen. 2011. A meta-analysis of crop pest and natural enemy response to landscape complexity. Ecol. Lett. 14: 922–932.

Daily, G. C., S. Polasky, J. Goldstein, P. M. Kareiva, H. A. Mooney, L. Pejchar, T. H. Ricketts, J. Salzman, and R. Shallenberger. 2009. Ecosystem services in decision making: time to deliver. Front. Ecol. Environ. 7: 21–28.

Rusch, A., R. Bommarco, M. Jonsson, H. G. Smith, and B. Ekbom. Flow and stability of natural pest control services depend on complexity and crop rotation at the landscape scale. J. Appl. Ecol. in press.

Schmidt, M. H., A. Lauer, T. Purtauf, C. Thies, M. Schaefer, and T. Tscharntke. 2003. Relative importance of predators and parasitoids for cereal aphid control. P. R. Soc. B Biological Sciences 270: 1905–1909.

Wiktelius, S. 1987. Distribution of *Rhopalosiphum padi* (Homoptera: Aphididae) on spring barley plants. Ann. Appl. Biol. 110: 1–7.

Östman, Ö., B. Ekbom, and J. Bengtsson. 2001. Landscape heterogeneity and farming practice influence biological control. Basic Appl. Ecol. 2: 365–371.

Östman, Ö., B. Ekbom, and J. Bengtsson. 2003. Yield increase attributable to aphid predation by ground-living polyphagous natural enemies in spring barley in Sweden. Ecol. Econ. 45: 149–158.

# Habitat manipulations to increase parasitism of leafrollers and broader landscape influences on the predator complex in apples

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Suppression of orchard pests by natural enemies is influenced by production and pest management programs internal to the orchards and by ecosystem services provided by surrounding habitats both near and far. While management programs can be greatly modified, changing surrounding habitat is more challenging and how to do so is a topic of debate. Integration of crop production and the preservation of non-crop habitats have become increasingly tenuous due to the steady increase of agricultural intensification at the expense of unmanaged habitats.

Two strategies have been proposed to promote agriculture while conserving non-crop habitats. The first approach, habitat sparing, protects unmanaged habitats by setting them aside while contiguous and large areas of production dominate other areas with little unmanaged habitat. Tscharntke et al. (2005, 2012) emphasize that land sparing reduces the likelihood of crops receiving ecosystem services provided by native habitats while advocates of land sparing suggest that it is more efficient for production and that ecosystem service providers (ESPs: predators, parasitoids, pollinators) are of negligible importance in intense agriculture. The contrasting approach is termed land sharing, where crop production is intermingled with unmanaged habitats (Green et al. 2005, Tscharntke et al. 2005, 2012).

The intermountain basins of central Washington are characterized by xeric shrub-steppe habitat (< 20 cm of precipitation/yr.) interspersed with rivers and narrow riparian zones giving way to oak then pine forests with increasing altitude and latitude. Tree fruit production depends on irrigation and benefits from the xeric habitat that reduces some disease and arthropod pests. However, the shrub steppe appears to produce significantly fewer ESPs than produced by riparian habitats (Rathman and Brunner 1988, Miliczky and Horton 2005). Several studies have shown that natural enemies are more abundant and diverse in crop edges near to non-crop source habitats or habitats specifically created to enhance natural enemies (Bianchi and Van Der Werf 2003, Corbett and Rosenheim, 1996, Miliczky and Horton 2005, Thies et al. 1997). This contrasts with broader landscape influences identified for provision of pollinators in the floor of the Central Valley of California (Kremen et al. 2004). The importance of proximity of natural habitats for arrival of ESPs to crops is likely to vary with ESP type including traits such as size and life style with large predators more able to colonize crops from distant habitats than small parasitoids (Tscharntke et al. 2005). However, these general rules may be confounded by differences in the ESP complex across landscape types. We feel that a detailed understanding of the phenology, habitat and host preferences, as well as many other traits of a specific ESP may be needed to optimize the ecosystem service it provides, much as is done in effort to conserve threatened species (Kremen et al. 2005).

Here we describe how naturally occurring or intentionally created rose habitat adjacent to orchards enhances ecosystem service of pest leafroller control by the parasitoid *Colpoclypeus florus* (Walker) (Hymenoptera: Eulophidae). We contrast that study with an evaluation of the influence of organic management practices and the nature of the broader landscape surrounding several apple orchards in central Washington on the natural enemy abundance and diversity within those orchards.

Thickets of the multifloral rose, *Rosa woodsi* Lindley (*Rosaceae*), are common in sub-riparian habitats in central Washington. It supports the non-pest (in this setting) leafroller, *Ancylis comptana* (Frölich) (Lepidoptera: Tortricidae), which overwinters as mature larvae in diapause. Overwintering as mature larvae is relatively rare among the Tortricidae but such a host is needed in the autumn for overwintering by *C. florus*. When rose thickets infested with *A. comptana* and *C. florus* were found near to orchards, we observed that the wasps entered orchards and attacked pest leafrollers (Pfannenstiel et al. 2012). Also, parasitism rates of the pest leafrollers in orchards by *C. florus* was seen to be negatively correlated with distance from the rose source in transect studies (Figures 1 and 2). The pattern of declining parasitism rates with distance from the riparian area along the Yakima River was observed for *C. florus* and for two additional parasitoids, *Oncophanes americanus* (Weed) (Hymenoptera: Braconidae), and tachinid flies(Diptera: Tachinidae) in a broad landscape study covering over 600 ha of apples, pears and cherries. The overwintering hosts and habitats use by *O. americanum* and the tachinids remains unclear (Pfannenstiel et al. 2012).

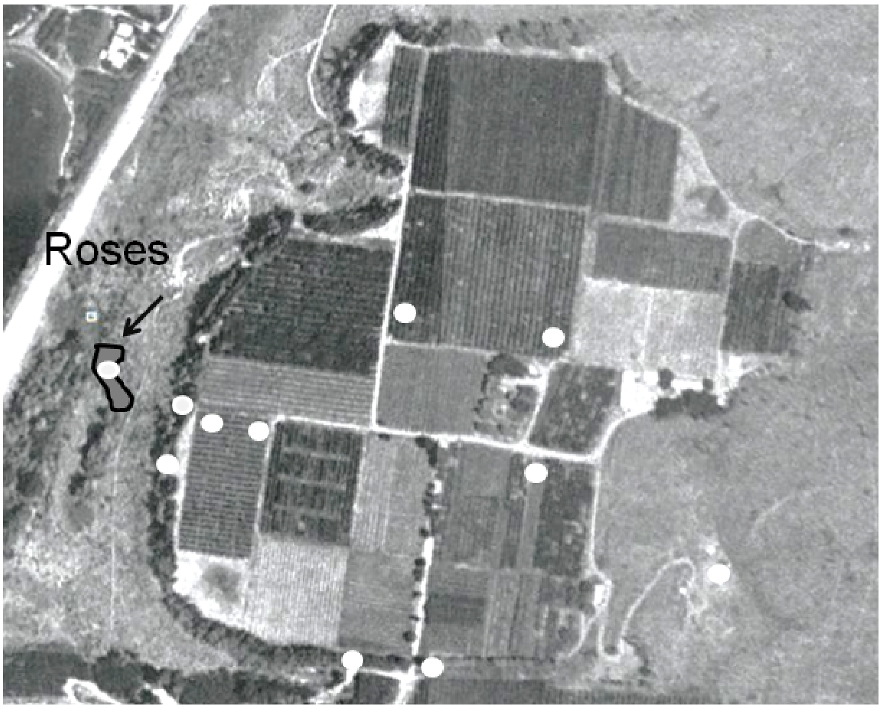

Fig 1. Experimental orchard (17 ha) adjacent to rose thicket (black outline) and sentinel leafroller deployment sites shown as white circles

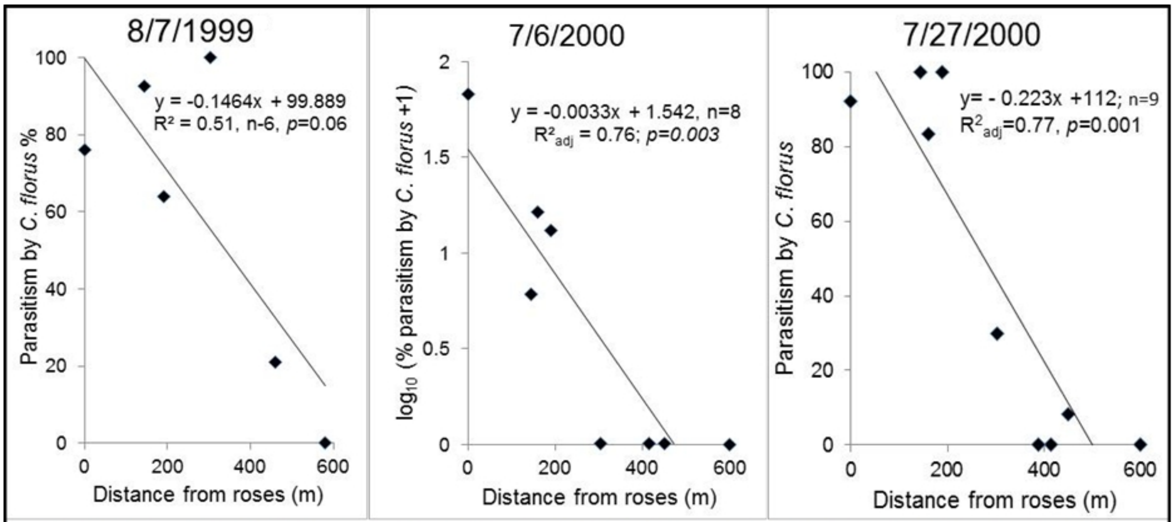

Fig 2. Percent parasitism by *C. florus* of sentinel hosts deployed at orchard sites showing decrease in parasitism with increasing distance from rose thicket (see Fig 1).

Our studies of naturally occurring rose thickets as habitats exporting ESPs, stimulated us to experimentally test if it could be engineered (Unruh et al. 2012). Three of four gardens of rose plus strawberry that were planted adjacent to orchards proved to support both *A. comptana* and *C. florus* and led to export of *C. florus* to adjacent orchard blocks in spring. One example of this result is depicted in Figure 3. In an attempt to further understand the behavior of *C. florus* entering orchards from a rose thicket, we used a protein marking technique wherein *C. florus* acquired the mark by walking on rose leaves dusted with soy flour. Detection of soy on wasps collected in sticky traps throughout the orchard demonstrated the wasps' origin and that they readily flew 100 m (see Figure 4). We conclude from these studies that creation of rose habitats can help increase parasitism of leafroller pests in fruit orchards in Washington. Orchardists concur and have planted more than 50 habitats throughout the state. Several plantings are being added each year, stimulated by support to growers to create habitats by the USDA, Natural Resources Conservation Service (Brewer et al. 2009, Unruh, personal observations).

The role of habitats more distant from orchards remains unclear. The statistical analysis of the effects of more distant (> 500 m) can easily be confounded by redundancy of the same habitats at distance or vice versa. A productive habitat type may be abundant in areas beyond 500 m from the target crop but a valuable parasitoid may prove incapable of entering the crop in adequate abundance due to low dispersal abilities associated with its size (Corbett and Rosenheim 1996, Kremen et al. 2005, Tscharntke et al. 2005, Pfannenstiel et al. 2012). In contrast, very small source areas that are very close to the crop may have strong positive effects if the ESP has a short generation time and can breed to great abundance once inside the crop.

Using weekly beat tray samples we identified predator species diversity, abundance and phenology at 9 orchards over 2 years (Horton et al. 2012). We used canonical correspondence analysis to measure the influence of surrounding (800 m radius) habitat composition and orchard management on community composition. Latitude, intensity of pest control, and two landscape variables (shrub steppe and row crops) appeared to be important explanatory variables. The unsprayed (no pest controls) sample sites (TFREC, Moxee) were significantly separated from the other sites. Certain ladybeetle species were more common northwards (*Adalia*, *Harmonia*), illustrating the importance of large geographic effects. Difficulties in untangling landscape effects from other factors can be illustrated by examining two ladybeetle taxa: *Chilocorus* and *Hyperaspis*. Both taxa were common at sites surrounded by sagebrush or annual cropping systems. *Hyperaspis* is a common inhabitant of sage systems (Acorn 2007). However, the orchards at which the taxa were abundant were also free of insecticidal controls and were found to have high densities of a preferred prey of both beetle taxa, namely San Jose scale. In sum, while we might infer that landscape factors affected composition of the ladybeetle community in orchards, it can be difficult to examine those effects in the absence of other potentially confounding factors.

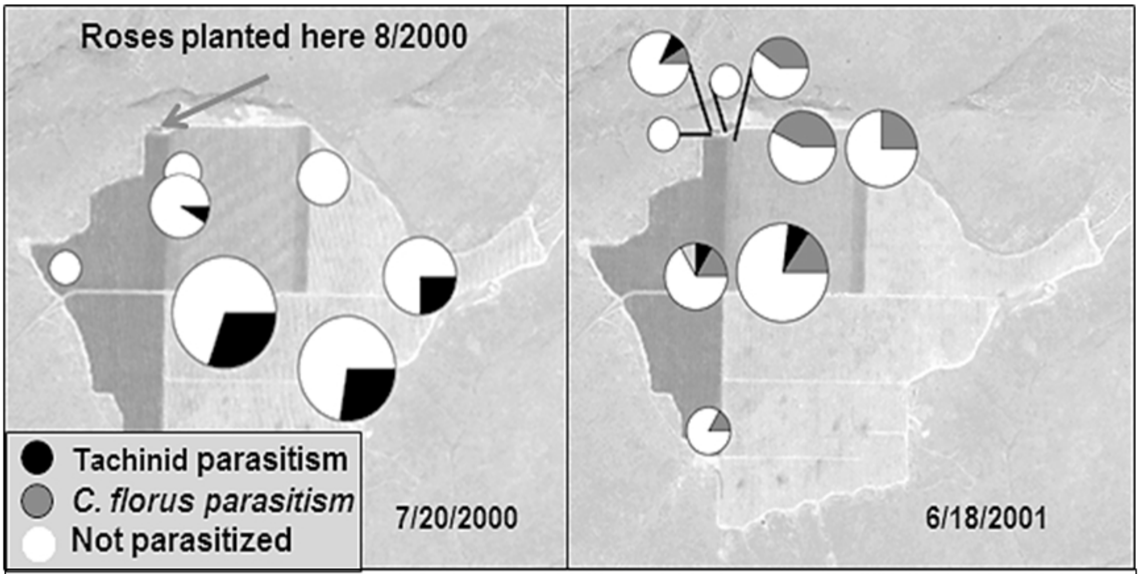

Fig 3. Parasitism (pie charts) of sentinel leafroller larvae deployed in 25 ha orchard before rose and strawberry habitat was planted and same site one year later when habitat harbored the leafroller *A. comptana,* a non-pest host for *C. florus* (dark grey pie slices)

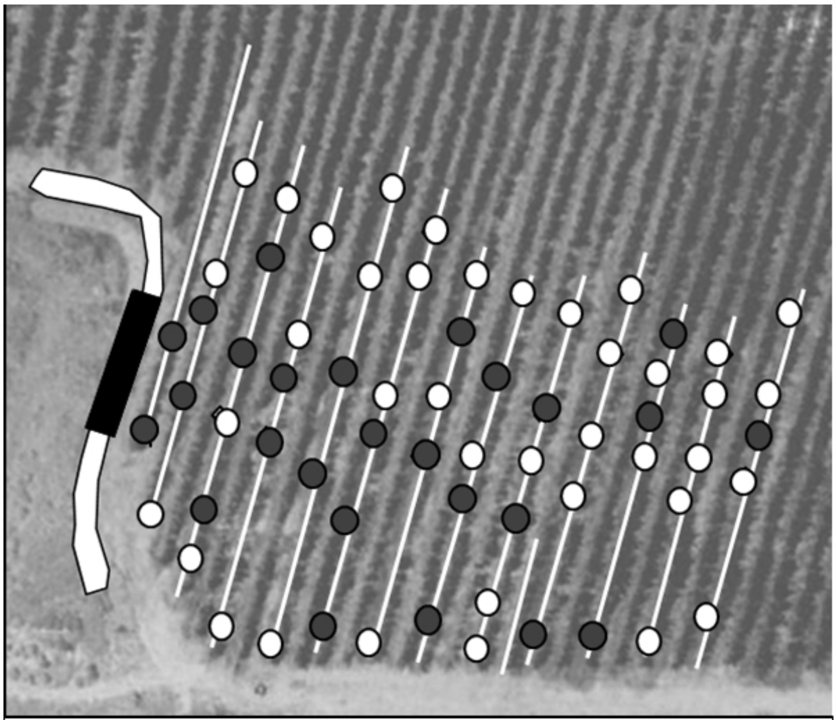

Fig 4. Capture sites of *C. florus* marked with soy flour (black circles) and other trap sites (white circles) which captured unmarked or no *C. florus* in orchard following treatments of a portion (black bar) of a rose thicket (white shaded area) with the soy flour marker.

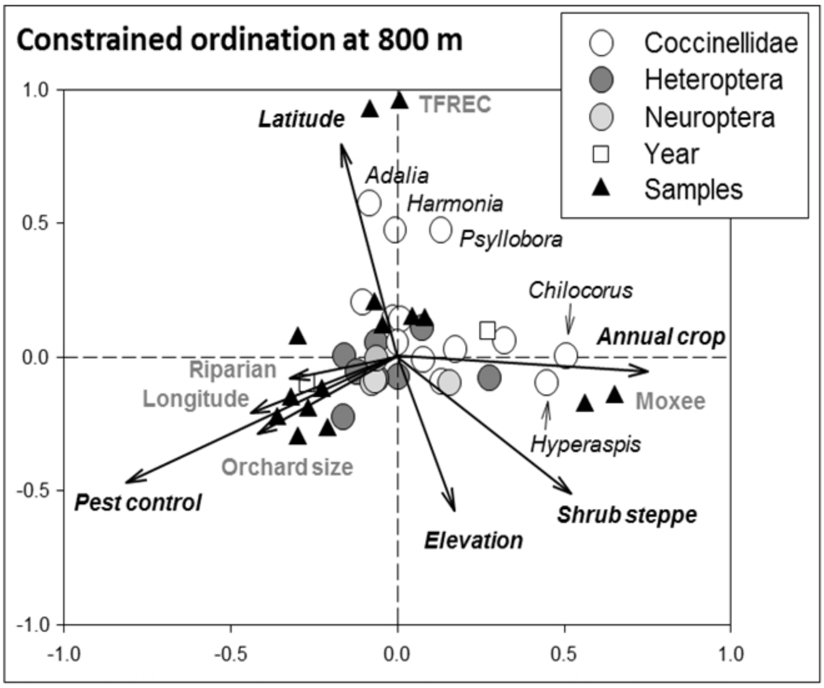

Figure 5. Canonical correspondence analysis plot which graphically depicts the influence of habitat composition in the surrounding 800 m (percent shrub/steppe, row crops, riparian), geographic measures (longitude, latitude, elevation), organic pest control programs (none, oil with lime sulfur, and oil with LS and spinosad), orchard size, year, and sample orchards on the abundance of three insect groups.

We see the broad landscape study as a vehicle to discover the habitat niche and needs of ESPs and how our management practices can influence these as well. When additional data on differences in phenology, abundance and time of appearance in orchards are compared, we have useful insights into which species we should focus studies. Those explorations must include better understanding of their overwintering habitats and other ecological needs of the ESPs of interest and what steps we can take to make in or near orchards to enhance their contributions to pest suppression.

## References

Acorn, J. 2007. Ladybugs of Alberta. Univ. of Alberta Press, Edmonton, AB, Canada.

Bianchi, F. J. J. A., and W. Van Der Werf. 2003. The Effect of the Area and Configuration of Hibernation Sites on the Control of Aphids by *Coccinella septempunctata* (Coleoptera: Coccinellidae) in Agricultural Landscapes: A Simulation Study. Environ. Entomol. 32: 1290–1304.

Brewer, M. J., E. G. Rajotte, J. R. Kaplan, P. B. Goodell, D. J. Biddinger, J.N. Landis, R. M. Nowierski, B. F. Smallwood, and M. E. Whalon. 2009. Opportunities, experiences, and strategies to connect integrated pest management to U.S. Department of Agriculture conservation programs. American Entomologist 55: 140–146.

Corbett, A., and J.A. Rosenheim. 1996. Impact of a natural enemy overwintering refuge and its interaction with the surrounding landscape. Ecol. Entomol. 21: 155–164.

Green, R. E., S. J. Cornell, P. W. Jörn Scharlemann, and A. Balmford. 2005. Farming and the fate of wild nature. Science, 307: 550–555.

Horton, D.R., E. Miliczky, V.P. Jones, C.C. Baker, and T.R. Unruh. 2012. Diversity and phenology of the generalist predator community in apple orchards of Central Washington State (Insecta, Araneae). Can. Entomol. 144: 691–710.

Kremen, C., N. M. Williams, R. L. Bugg, J. P. Fay, and R. W. Thorp. 2004. The area requirements of an ecosystem service: crop pollination by native bee communities in California. Ecol. Lett. 7: 1109–1119.

Kremen, C. 2005. Managing ecosystem services: what do we need to know about their ecology? Ecol. Lett. 8: 468–479.

Miliczky, E. R., and D. R. Horton. 2005. Densities of beneficial arthropods within pear and apple orchards affected by distance from adjacent native habitat and association of natural enemies with extra-orchard host plants. Biol. Control 33: 249–259.

Pfannenstiel, R., B. Mackey, and T. Unruh. 2012. Leafroller parasitism across an orchard landscape in central Washington and effect of neighboring rose habitats on parasitism. Biol. Control. 62: 152–161

Rathman, R. J., and J. F. Brunner. 1988. Abundance and composition of predators on young apple, *Malus domestica* Borkhausen, within sagebrush and riparian species pools in north central Washington. Melanderia 46: 66–81.

Thies, C., C. Denys, T. Tscharntke and B. Ulber. 1997. Influence of field margins and fallowland on pest-benifical insect-interactions on oilseed rape (*Brassica napus* spp. *oleifera*). Verh Ges Ökol., 27: 393–398.

Tscharntke, T., A. M. Klein, A. Kruess, I. Steffan-Dewenter, and C. Thies. 2005. Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. Ecol. Lett. 8: 857–874.

Tscharntke,T. Y. Clough, T. C. Wanger, L. Jackson, I. Motzke, I. Perfecto, J. Vandermeer, and A. Whitbread. 2012. Global food security, biodiversity conservation and the future of agricultural intensification, Biol. Conserv. 151: 53–59.

Unruh, T.R., R. S. Pfannenstiel, C. Peters, J. Brunner, and V. P. Jones, 2012. Parasitism of leafrollers in Washington fruit orchards is enhanced by perimeter plantings of rose and strawberry. Biol. Control 62: 162–172.

# Conservation of native coccinellids and biological control of aphids in alfalfa: a landscape perspective

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## Introduction

Conservation of native species needs an innovative approach. Nowadays, natural reserves are necessary but not enough for this purpose and therefore biodiversity conservation should assume a landscape perspective, including more disturbed habitats like agroecosystems. It is well known that as compositional and configurational heterogeneity of agricultural landscapes increases so does animal biodiversity (Fahrig et al. 2011), including natural enemies of pests. Nevertheless, the current trend toward simplification of agricultural landscapes, and the associated loss of some perennial natural or semi-natural structures may decrease natural enemy's diversity. This, in turn, could result in a lower biological control service within a given crop, because of the loss of native species with potentially complementary niches that no longer will colonize the crop from the surroundings. Also, it could be possible that the best biological control occurs when only a few generalist vagile efficient species are present in the landscape (Tscharntke et al. 2007). If these species are exotics, then preserving habitat for native species could interfere with biological control.

Coccinellids (Coleoptera: Coccinellidae) are important natural enemies in alfalfa fields in central Chile. Their assemblages are composed of about 20 species, both natives and exotics. A recent study has shown that the proportion of native individuals and species are higher in less disturbed habitats (Grez et al. in press). Also, within this assemblage, some native species are more abundant in alfalfa fields adjacent to perennial edges, like hedgerows and woodlands (Grez et al. 2010). But how the composition and heterogeneity of the landscape -at larger scales- affect the composition and abundance of native and exotic coccinellids in alfalfa and pest biological control it is unknown.

In this study, we evaluated the effects of landscape composition and heterogeneity -at different spatial scales- on the diversity and abundance of native and exotic coccinellids, associated with alfalfa fields, and also on biological control service (BSI).

## Material & Methods

The study was carried out at Pirque, near Santiago, Chile, in 2010–2012 growing seasons. This is an agricultural landscape dominated by orchards and annual crops but also having some perennial semi-natural vegetation and native vegetation between crops that confers heterogeneity. Within this area, we selected 10 non-overlapping landscapes, where compositional and configurational heterogeneity were characterized at three spatial scales (1000, 500 and 250 m from a focal alfalfa field). We classified the cover types into 12 categories, including annual crops, orchards and vineyards, semi-urban areas, alfalfa fields and native shrublands, among others, and calculated their area in each landscape to determine landscape composition. Also with these data, we calculated the compositional heterogeneity by Simpson's diversity Index, and the configurational heterogeneity through patch size, edge density and shape index using Patch Analyst of ArcGIS 9.3 ®

Coccinellids and aphids were sampled in each of the 10 focal alfalfa fields, in spring, summer and autumn 2010-2011. Coccinellids were sampled during two weeks by placing 20 sticky yellow traps. Aphids were also sampled in each field. Cover types were reduced through PCA (at 1000, 500 and 250 m). The first two principal components (PC1 and PC2) accounted for over 70% of the variance, so analyses were restricted to these two components. Akaike's Information Criterion, adjusted for a small sample size ($AIC_c$), $R^2$ and parsimony criteria, were used as a model selection procedure to examine the influence of landscape composition (PC1 and PC2), compositional heterogeneity (Simpson's diversity), configurational heterogeneity (mean patch size, edge density and mean shape index) and aphid abundance on coccinellid assemblages in alfalfa (total abundance, exotic and native abundance, proportion of natives and diversity).

In spring 2012, BSI was estimated following Gardiner et al. (2009a), by experimentally excluding or allowing natural enemy's access to pea aphid infested plants and comparing aphid population growth at 7 and 14 days. Aphids and coccinellids abundance in the alfalfa fields were also estimated. To test the relationships among landscapes attributes and BSI the same procedure described above was used.

## Results

In total, almost 9000 coccinellids were collected in alfalfa in 2010-2011 season, of which 84% were exotics, belonging to 6 species, with the most abundant species being *Hippodamia variegata* (Goeze) and *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). Among the native species, the most common was *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae). The only significant relations between landscape and coccinellid variables were found at 1000 m. At this scale, PC1 was positively correlated with orchards and vineyards, while negative loadings were correlated with annual crops. Consequently, sites with positive values of PC1 had a high proportion of orchards and vineyards, while sites with negative values of PC1 had a high proportion of annual crops. For the PC2, sites with positive loadings were correlated with annual crops and negatively loadings were correlated with the shrublands and semi-urban covers. Sites with positive values of PC2 had a high proportion of annual crops, while sites with negative values of PC2 had a high proportion of shrublands and semi-urban covers. Landscape surrounding alfalfa crops differentially influenced the composition of native and exotic coccinellid assemblages within this crop, as it has been observed also in other agroecosystems (Gardiner 2009b). Compositional (Simpson) or configurational heterogeneity rarely were significant predictors of the coccinellid assemblages within alfalfa fields. Only patch shape index related negatively with the proportion of native coccinellids suggesting that landscapes with more irregular patches favor native over exotic coccinellids in alfalfa. Although total abundance of coccinellids was not affected by landscape composition, the abundance of native coccinellids in alfalfa was lower in landscapes with more annual crops and higher in landscapes with more shrublands and semi-urban covers (PC2). Similarly, the proportion of native coccinellids over exotic ones in alfalfa was lower in landscapes with more annual crops and higher in those with more perennial covers, like orchards and vineyards and the shrublands (PC1 and PC2). Accordingly, coccinellid diversity was also lower in alfalfa fields in landscapes with more annual crops (PC2).

In spring 2012, BSI at 7 days was significantly related with mean patch shape and landscape composition, being higher when landscapes were composed of more regular patches and dominated by annual crops, and when aphid densities were lower. At 14 days, BSI was associated negatively with aphid density at 7 days and patch shape, with a more efficient biological control at lower aphid density and landscapes with more regular patches. Also, BSI in spring was positively related with the total abundance of coccinellids (total) and exotic, but not with natives.

Thus, when considering the whole season, landscapes with regular patches and more annual crops negatively affect the abundance and proportion of native coccinellids in alfalfa, suggesting that for conserving native species in this crop, it would be necessary to promote landscapes with more irregular patches and less annual crops. Nevertheless, BSI in spring is greater in landscapes with regular patches and with more annual crops, and it is not associated with the abundance of native coccinellids. Instead BSI in spring is higher when exotic coccinellids are more abundant. This contradiction may be explained by the differential time of colonization of native and exotic coccinellids, with exotics arriving early in spring, while natives increasing later in the season. Therefore, for a more comprehensive understanding of the role of landscape on conservation of native coccinellids and biological control, it will be necessary to gather the data for the whole season.

## Acknowledgements

We are very grateful to FONDECYT 1100159 and the LEAF team.

## References

Fahrig, L., J. Baudry, L. Brotons, F. G. Burel,T. O. Crist, R. J. Fuller, C. Sirami, G. M. Siriwardena, and J. Martin. 2011. Functional landscape heterogeneity and animal biodiversity in agricultural landscapes. Ecol. Lett. 14: 101–112.

Gardiner M. M., D. A. Landis, C. Gratton, C. D. Difonzo, M. O’Neal, J. M. Chacon, M. T. Wayo, N. P. Schmidt, E. E. Mueller, and G. E. Heimpel. 2009a. Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. Ecol. Appl. 19: 143–154.

Gardiner, M. M., D. A. Landis, C. Gratton, N. Schmidt, M. O'Neal, E. Mueller, J. Chacon, G. E. Heimpel, and C. D. Di Fonzo. 2009b. Landscape composition influences patterns of native and exotic lady beetle abundance. Divers. Distrib. 15: 554–564.

Grez, A. A., C. Torres, T. Zaviezo, B. Lavandero, and M. Ramírez. 2010. Migration of coccinellids to alfalfa fields with varying adjacent vegetation in Central Chile. Ciencia e Investigación Agraria 37: 111–121.

Grez A. A., T. A. Rand, T. Zaviezo, and F. Castillo-Serey. 2013. Land-use intensification differentially benefits alien over native predators in agricultural landscape mosaics. Divers. Distrib. (in press).

Tscharntke T., R. Bommarco, Y. Clough, T. O. Crist, D. Kleijn, T. A. Rand, J. M. Tylianakis, S. van Nouhuys, and S. Vidal. 2007. Conservation biological control and enemy diversity on a landscape scale. Biol. Control 43: 294–309.

# Session 13: Functional Implications of Non-Prey Feeding in Biological Control

## On the interplay between omnivores' behavior and the nutritional value of plant and prey foods

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Consumers require specific balance of nutrients to survive, develop and reproduce. Most insect species are nutritionally-imbalanced foods, forcing their predators to regulate nutrient intake to match their physiological requirements. Omnivory could be one of the possible mechanisms for nutrient regulation.

Many natural enemies used in biological control programs are true omnivores that supplement their prey-rich diet with plant materials. We examined the nutrient composition of plant and prey foods and their suitability for the omnivorous mite, *Amblyseius swirskii* Athias-Henriot (Mesostigmata: Phytosiidae), and tested whether switching behavior, i.e. omnivory, is used to regulate nutrient intake and increase omnivore's fitness.

We examined the fecundity of females when fed pepper pollen or prey found in pepper crops in the Arava valley, Israel (*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), *Tetranychus utricae* Koch (Trombidiformes: Tetranychidae) and *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)). Additionally, females were offered pollen from two other plants, *Typha* sp. (*Typhaceae*) and *Zea mays* L. (*Poaceae*). These pollens could serve as possible food supplements for the omnivorous mite in biological control programs. Results show that females deposited more eggs when fed *Typha* pollen than any of the prey species. Egg deposition was significantly higher on *Z. mays* pollen than on *T. utricae* eggs or *F. occidentalis* nymphs. Finally, reproduction was lowest on pepper pollen. These results suggest that the nutrient compositions of the tested prey and of pepper pollen are inadequate for mite reproduction.

To identity the nutrients that render these prey unbalanced for the mites, we explored their chemical compositions. Nutritional analyses revealed that overall, pollen has a higher sugar content (23-40%) than the prey (0.7-2.2%). In contrast, percentage of protein is higher in prey (~50%) than pollen (16-20%). We hypothesized that the recorded discrepancies in sugar and protein content encourage the omnivore to switch between pollen and prey in order to balance its nutrient intake.

The benefit of such switching was tested by offering female mites that were fed a pre-treatment diet of *Typha* pollen or *F. occidentalis* larvae for 5 days, the same or the alternative food for 4 additional days. We found that switching from plant to animal food and vice versa resulted in a higher female fecundity than consuming the same diet without switching. The contribution of diet switching to female fecundity was especially strong when females were switched from prey to pollen diets. It appears therefore that switching between food sources allows omnivores to regulate their nutrient intake and thus achieve higher performance. We were interested therefore in assessing how nutrient balancing may influence omnivore-prey population dynamics, especially considering the commonly-found variations in the availability of foods of different nutritional values.

Most animals forage in nutritionally complicated environments in which the densities of complementary foods vary in space and time. Locally rare foods can lead to nutritional deficiencies in consumers, yet there has been little consideration of the influence of nutritional deficits, and consumers' efforts to redress these deficits, on population and community dynamics. Consumers can redress accumulated deficits using nutrient-specific foraging, defined here as the ability of an animal to bias its foraging effort towards certain foods on the basis of its own nutritional state. We used an ordinary differential equation model to show that nutrient-specific foraging may destabilize omnivore–prey population dynamics, depending on the degree to which the omnivore alters its foraging in an attempt to balance its nutritional state. Overall, the model is characterized by a causal loop between the omnivore's nutritional state, food preferences and population dynamics. Consequently, the omnivore exhibited a dynamic functional response, in which the relationship between prey density and omnivore per capita consumption of prey was influenced by the current nutritional state of the omnivore. With regards to omnivory in general, our results reinforce the idea that statements about the effects of omnivory on population or community stability are not possible without a consideration of the behavioral mechanisms of diet mixing.

# Diet switching and mixing in arthropod predators: Consequences for digestion, development and biological control

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Among arthropod predators, omnivory is frequent, involving in addition to a variety of prey, consumption of non-prey foods such as pollen, nectar (floral and extrafloral), honeydew, fungi, and plant sap or parts (Coll and Guershon 2002, Lundgren 2009). By definition, omnivory entails diet mixing and diet switching or shifting over time, based on availability and selection of food items by the individual generalist arthropod (Singer and Bernays 2003, Chambers et al. 1998, Behmer 2009). In the field, plants provide food and other resources which frequently mediate interactions among omnivores and their prey and non-prey foods (Eubanks and Styrsky 2005). Resource pulses (Yang et al. 2008) such as pollen shed (Lundgren and Wiedenmann 2004) and specific prey populations (e.g., aphids and insect eggs) provide plentiful but ephemeral resources (Michaud and Jyoti 2008). As a result, individual predators likely have to shift their diets over their adult lives, and may encounter a huge range of potential food items, particularly if the predator moves from habitat to habitat. However, there has been little research into diet switching, its consequences in arthropod predator physiology, reproduction, dispersal, and implications for biological control.

*Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae), a common and widespread omnivore in the New World, consumes diverse insect prey and non-prey foods, and populations alter their reliance on selected prey items when alternative foods (e.g., pollen or other prey types) become available (Hazzard and Ferro 1991, Cottrell and Yeargan 1998, Lundgren et al. 2004). These variable spatiotemporal patterns of food availability select for mobility and/or physiological adaptability to ingestion and digestion of a wide range of foods. Quality or value of the different foods available to omnivores, however, depends on their ability to effectively digest them, which in turn may depend on the pattern of diet switching and mixing: the order and duration in which different foods are encountered and ingested. We decided to test a diet switch which most likely occurs frequently in the field with *C. maculata*, involving insect eggs (of Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae)) and maize, *Zea mays* L. (*Poaceae*) pollen, for its effect on rapidity of digestion as measured by diet-specific quantitative PCR (Weber and Lundgren 2011). Switches using adult male and female beetles, in the direction from eggs to pollen, and also from pollen to eggs, were compared also to digestion (disappearance of DNA marker over 8 hours) in non-switched beetles fed on pollen or eggs exclusively as adults. Beetles which were switched from eggs to pollen experienced a significantly slower digestion, as measured by qPCR, than any of the other three treatments. This demonstrates an asymmetric response to diet switching, in which the omnivorous insect is unable to digest pollen after a period of feeding on prey eggs, whereas there is no such lag in the converse switch. However, it was not just the novelty of the diet, but the previous diet, that influenced speed of digestion. In another experiment (Weber and Lundgren 2011), male and female *C. maculata* pre-fed for 7 days on sucrose digested the insect eggs and maize pollen at a comparable rate to diet-experienced adults. The physiological basis for the apparent negative dietary conditioning of prey diet (insect eggs), is not known, and could arise from enzymes originating with the insect, its gut flora, and/or the food itself.

In another test with qPCR-based measurement of digestion of a single *L.decemlineata* egg, *C. maculata* adults were immediately afterwards fed a "chaser diet" of either no food, *ad libitum* apterous adults of potato aphid (*Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae))*,* or *ad libitum C. maculata* eggs. In this case the consumption of aphids promoted digestion of original prey which was significantly more rapid than with starvation; a cannibalistic diet was intermediate and not significantly different from either (Weber and Lundgren 2009). Thus certain diet mixes (or rapid switches) may promote more rapid nutrient assimilation.

A subsequent study (Weber unpublished data) tested switched and unswitched larval diets in *C. maculata*, using a yeast-based pollen substitute and insect (*Ephestia kuhniella* Zeller (Lepidoptera: Pyralidae)) eggs as the two diets. During the ~3-wk larval period, diets were switched (or unswitched) weekly between these two diets, for a total of 8 (=$2^3$) treatments (permutations), in addition to a mixed diet throughout the larval period. The yeast-based food for the first week resulted in higher mortality and longer developmental period to adult, and had the same effect in the second week, but only for those larvae which had been feed yeast in the first week. Older larvae successfully develop to adulthood on eggs or yeast, with diet effects of the third week unique to prior diet pattern. Thus there was a stage-specific effect of diet-switching within the larval period. This result is consistent with the earlier results of Lundgren and Weber (2010), in which early-instar *C. maculata* were less efficient at using non-prey resources (yeast and maize pollen).

The majority of studies on diet switching have focused on vertebrates and herbivorous insects (see citations in Weber and Lundgren 2011). But for effective biological control by generalist or omnivorous arthropod predators, knowledge of effects of prior diet, and of diet switching, is essential to the optimal functional and numeric response of biocontrols for conservation, augmentative, and inundative approaches. How do environmental variables such as temperature affect diet mixing (Miyasaka and Genkai-Kato 2009)? What role does the diversity of insect gut flora play (Colman et al. 2012)? How does life history plasticity, even including additional larval instars (Lee et al. 2012) influence response to diet switching? In a controlled rearing, as with the carbohydrate-loading tactics of human distance runners (Kerksick et al. 2008), could a regimen of diet-switching during rearing be devised to prepare predators for release, effective searching and consumption of targeted prey types? Could provision and withholding of specific food types in the field could promote more effective predation by omnivorous predators, either by supplementing needed resources when absent in the field, or by using removal of alternative foods to drive predators to attack target prey? More generally, how can biological control practitioners create or manipulate resource pulses (Yang et al. 2010) under controlled and also under field conditions, to encourage numerical and functional response of biological controls?

## References

Behmer, S. T. 2009. Insect herbivore nutrient regulation. Ann. Rev.Entomol. 54:165-187.

Bernays, E. A., M. S. Singer, and D. Rodrigues. 2004. Foraging in nature: foraging efficiency and attentiveness in caterpillars with different diet breadths. Ecol. Entomology 29: 389–397.

Chambers, P. G., D. Raubenheimer, and S. J. Simpson. 1998. The functional significance of switching interval in food mixing by *Locusta migratoria*. J. Insect Physiol. 44: 77–85.

Coll, M., and M. Guershon. 2002. Omnivory in terrestrial arthropods: mixing plant and prey diets. Ann. Rev. Entomol. 47: 267–297.

Colman, D. R., E. C. Toolson, and C. D. Takacs-Vesbach. 2012. Do diet and taxonomy influence insect gut bacterial communities? Mol. Ecol. 21: 5124–5137.

Cottrell, T. E., and K. V. Yeargan. 1998. Effect of pollen on *Coleomegilla maculata* (Coleoptera: Coccinellidae) population density, predation, and cannibalism in sweet corn. Environ. Entomol. 27: 1402–1410.

Eubanks, M. D., and J. D. Styrsky. 2005. Effects of plant feeding on the performance of omnivorous "predators". pp. 148–177 *In* F.L. Wackers, P.C.J. van Rijn and J. Bruin (Eds.) Plant-provided foods for carnivorous insects ). Cambridge University Press, Cambridge, UK.

Hazzard, R. V., and D. N. Ferro. 1991. Feeding responses of adult *Coleomegilla maculata* (Coleoptera: Coccinellidae) to eggs of Colorado potato beetle (Coleoptera: Chrysomelidae) and green peach aphids (Homoptera: Aphididae). Environ. Entomol. 20: 644-651.

Kerksick, C., T. Harvey, J. Stout, B. Campbell, C. Wilborn, R. Kreider, D. Kalman, T. Ziegenfuss, H. Lopez, J. Landis, J. L. Ivy, and J. Antonio. 2008. International Society of Sports Nutrition position stand: Nutrient timing, Journal of the International Society of Sports Nutrition **5**: 17 [doi:10.1186/1550-2783-5-17].

Lundgren, J. G. 2009. Relationships of natural enemies and non-prey foods. Springer, London, UK, 453 pp.

Lundgren, J. G., A. A. Razzak, and R. N. Wiedenmann. 2004. Population responses and food consumption by predators *Coleomegilla maculata* and *Harmonia axyridis* (Coleoptera: Coccinellidae) during anthesis in an Illinois cornfield. Environ. Entomol. 33: 958–963.

Lundgren, J. G., and D. C. Weber. 2010. Changes in digestive rate of a predatory beetle over its larval stage: implications for dietary breadth. J. Insect Physiol. 56: 431-437.

Lundgren, J. G. and R. N. Wiedenmann. 2004. Nutritional suitability of corn pollen for the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae). J. Insect Physiol. 50: 567–575.

Michaud, J. P. and J. L. Jyoti. 2008. Dietary complementation across life stages in the polyphagous lady beetle *Coleomegilla maculata*. Entomol. Exp. Appl. 126: 40-45.

Miyasaka, H and M. Genkai-Kato. 2009. Shift between carnivory and omnivory in stream stonefly predators. Ecol. Res. 24:11–19.

Singer, M. S. and E. A. Bernays. 2003. Understanding omnivory needs a behavioral perspective. Ecology 84: 2532-2537.

Weber, D. C., and J. G. Lundgren. 2009. Detection of predation using qPCR: effect of prey quantity, elapsed time, chaser diet, and sample preservation on detectable quantity of prey DNA. J. Insect Sci. 9: 41.

Weber, D. C., and J. G. Lundgren. 2011. Effect of prior diet on consumption and digestion of prey and non-prey food by adults of the generalist predator *Coleomegilla maculata*. Entomol. Exp. Appl. 140: 146–152.

Yang, L. H., J. L. Bastow, K. O. Spence, and A. N. Wright. 2008. What can we learn from resource pulses? Ecology 89: 621–634.

Yang, L. H., K. F. Edwards, J. E. Byrnes, J. L. Bastow, A. N. Wright, and K. O. Spence. 2010. A meta-analysis of resource pulse–consumer interactions. Ecol. Monogr. 80: 125–151.

# Functional biodiversity: How to make non-crop vegetation work for biological control

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In spite of increasing intensification of pest control over the last decades, on-field crop losses due to pests, diseases and weeds increased from 35% to 42% in the period of 1965 to 1990, (Pimentel 1991, Lewis et al. 1997). The continuing problems farmers face in terms of crop protection can be attributed to several factors. One reason lies in the fact that the use of non-selective agro-chemicals undermines natural crop protection mechanisms by eliminating beneficial organisms responsible for natural pest and disease control. The impact of beneficial organisms responsible for biological control is also severely constrained by land-use change. In the increasingly intensified agricultural landscape beneficial insect groups and the services they provide have declined as a result of the rapid depletion of semi-natural habitat and non-crop vegetation (Thies and Tscharntke 1999, Potts et al. 2010). A broad range of biological control agents depends on these semi-natural habitats for shelter, overwintering and as a source of nectar and pollen (Wäckers et al.2005, Lundgren 2009). Recent studies have provided clear evidence that natural enemies are indeed starving in the absence of flowering vegetation (Wäckers and Steppuhn 2003, Olsen and Wäckers 2007) and that this has dramatic consequences for natural pest control (Winkler et al. 2006).

## How can landscape management support natural pest control?

Insect predators that naturally occur in agricultural systems can be preserved and enhanced with simple cultural techniques (Conservation Biological Control). Such practices may involve provisioning natural enemies with resources that are lacking within the agricultural crop, such as nectar, pollen, alternative prey, or shelter (overwintering sites).

Adding floral resources can be a simple and effective tool to support predators and parasitoids and to harness the biological control services they provide. Winkler et al. (2006) showed that the majority of *Diadegma semiclausum* failed to attack any *Plutella xylostella* larvae in semi-field cages lacking nectar plants, whereas individuals provided with a nectar plant parasitized more than 300 larvae each. Thus, adding food sources to agro-ecosystems could be a simple and effective way to enhance the effectiveness of biological control programs.

The realization that flowering vegetation can be a powerful tool to support pollination and biological pest control has given rise to an increasing offering of commercial seed mixtures. Several countries provide subsidies for the use of such seed mixtures in field borders, non-crop areas or undergrowth. Despite the substantial investments in these programs, there is often surprisingly little information available with regard to the actual suitability of the included plants as food sources for the target insects (Wäckers 2004) or the ultimate impact of flowering plants on biological control (Heimpel and Jervis 2005).

## How to optimize pest control through targeted use of biodiversity in landscape management?

The creation of wildflower strips and management of other non-crop vegetation has emerged as a key tool to support beneficial insect groups and the ecosystem services they deliver. Yet, the efficacy of landscape management programs in terms of delivering ecosystem services is often limited by our incomplete understanding of the resource requirements of beneficial insects and how best to provide these resources.

The choice of field margin compositions for the support of natural pest control has long been done more or less arbitrarily (Gurr et al. 2005). These ´shotgun approaches´ have been ´hit and miss´ in terms of their delivery of ecosystem services. An uninformed choice of non-crop vegetation not only means missing out on potential benefits but may also actually generate negative effects. Arbitrarily composed floral vegetation can increase pest populations (Wäckers et al. 2007, Winkler et al. 2010), support enemies of beneficial insects (Araj et al. 2008), and cause weed or disease problems.

However, these problems can be avoided by taking a targeted approach in the choice of flowering plants for field margins or other types of non-crop vegetation. This targeted approach is based on the simple concept that different insect groups exploit different flowering plants. By selecting those plants that are especially suitable for the insects delivering pest control, while excluding plants that are preferred by nectar/pollen feeding pests, the positive impact of flowering landscape elements can be maximized (Wäckers and van Rijn 2012). On the basis of extensive recent work it is now possible to provide farmers with accurate prescriptions for seed mixes and landscape management that specifically target and optimize pest control benefits, while minimizing possible negative effects. As an alternative to the ´hit and miss´ approach, this ´targeted approach` is based on three key steps: 1) quantify the nectar or pollen bottleneck (level of food limitation of insect predators and parasitoids) under field conditions, 2) informed selection of flowering plants that can be successfully exploited by the target beneficial, and ideally not by pests 3) provide the flowering plants in ways (spatial and temporal patterns) that optimize benefits to beneficial delivering natural pest control services.

The use of this targeted selection of flowering plants has proven to generate significant benefits to natural pest control in a number of large scale projects worldwide (e.g www.ecostac.co.uk). This approach not only helps enhance natural populations of insect predators or parasitoids (so-called conservation biological control). Suitable flowering plants can also improve establishment when releasing commercially available biocontrol organisms (augmentative biological control). In the latter case, the flowering vegetation will not only help feed the released insects, it will also help retain them in the area of release.

By supporting the beneficial insects that deliver pest control services through targeted landscape management, the need for chemical intervention can be drastically reduced. This means less mortality of the naturally occurring predators and pollinators, thus generating a positive feed-back loop in terms of both pest control and pollination.

## Focus on the farmer

By focusing on the farmer´s core business of crop production and by including farmers and their concerns in the development of the margins, real and broad engagement of the farming community can be realized. This enhances the involvement of farmers and increases the number of farmers willing to utilize higher level agro-environment schemes, again creating benefits to overall biodiversity.

## References

Araj, S., S. Wratten, A. Lister, and H. Buckley. 2009. Adding floral nectar resources to improve biological control: Potential pitfalls of the fourth trophic level. Basic Appl. Ecol. 10: 554–562.

Constanza, R., R. d’Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. O’Neill, J. Paruelo, R. G. Raskin, P. Sutton, and M. van den Belt. 1997. The value of the world’s ecosystem services and natural capital. Nature. 387: 253–260.

Gurr, G. M., S. D. Wratten, J.Tylianakis, J. Kean, and M. Keller. 2005. Providing plant foods for insect natural enemies in farming systems: balancing practicalities and theory. pp. 326–347. In F. L. Wäckers, P. van Rijn, and J. Bruin (eds.), Plant-provided food for carnivorous insects: A protective mutualism and its applications . Cambridge University Press, Cambridge, UK.

Heimpel, G. E., and M. A. Jervis. 2005. An evaluation of the hypothesis that floral nectar improves biological control by parasitoids. pp. 267-304. In F. L. Wäckers, P. van Rijn and J. Bruin (eds.), Plant-provided food for carnivorous insects: a protective mutualism and its applications. Cambridge University Press, Cambridge, UK.

Lewis W. J., J. C. van Lenteren, S. C. Phatak, and J. H. Tumlinson. 1997. A total system approach to sustainable pest management. P. Natl. Acad. Sci. USA 94: 12243–12248.

Lundgren, J.G. 2009. Relationships of natural enemies and non-prey foods. Springer, Berlin.

Olson, D., and F. L. Wäckers. 2007. Management of field margins to maximize multiple ecological services. J. Appl. Ecol. 44:13–21.

Pimentel, D. (ed.). 1991. CRC Handbook of Pest Management in Agriculture, Vol. 1, 2nd Edition. CRC Press, Boca Raton, FL.

Potts, S. G., J. C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, and W. E. Kunin. 2010. Global pollinator declines: Trends, impacts and drivers. Trends Ecol. Evol. 25: 345–353.

(TEEB) The Economics of Ecosystems and Biodiversity. 2010. The Economics of Ecosystems and Biodiversity: The ecological and Economic Foundations. Earthscan, London.

Thies, C., and T. Tscharntke. 1999. Landscape structure and biological control in agroecosystems. Science 285: 893–895.

Wäckers, F. L., and A. Steppuhn. 2003. Characterizing nutritional state and food source use of parasitoids collected in fields with high and low nectar availability. IOBC wprs Bulletin 26: 203–208.

Wäckers, F. L. 2004. Assessing the suitability of flowering herbs as parasitoid food sources: Flower attractiveness and nectar accessibility. Biol. Control 29: 307–314.

Wäckers, F. L., J. Romeis, and P. C. J. van Rijn. 2007. Nectar and pollen-feeding by insect herbivores and implications for multitrophic interactions. Annu. Rev. Entomol. 52: 301–325.

Wäckers, F. L., and P. C. J. van Rijn. (2012). Pick and mix: selecting flowering plants to meet requirements of target biological control insects. pp. 139–165. In G. Gurr (ed.), Biodiversity and insect pests. Wiley Blackwell, Oxford, UK.

Wäckers, F. L., P. C. J. van Rijn, and J. Bruin (eds.). 2005. Plant-provided food for carnivorous Insects: a protective mutualism and its applications. Cambridge University Press, Cambridge, UK.

Winkler, K. F. L. Wäckers, A. J. Termorshuizen, and J. C.van Lenteren. 2010. Assessing potential risks and benefits of floral supplements in conservation biological control. BioControl 55: 719–727

Winkler, K., F. L.Wäckers, G. Bukovinski-Kiss, and J. C. van Lenteren. 2006. Nectar resources are vital for *Diadegma semiclausum* fecundity under field conditions. Basic Appl. Ecol. 7:133–140.

# Decoding the diets of spiders: non-prey resources for dietary supplementation

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Generalist predators have long been viewed as acting in a positive manner in biological control (reviewed by Sunderland et al. 1997, Symondson et al. 2002, Welch et al. 2012), yet their role in biologically meaningful pest suppression has been controversial (Hagen and Van den Bosch 1968, Symondson et al. 2002). This is because they feed on a diverse array on non-pest foods, sometimes preferentially (Bilde and Toft 1997), that can ultimately reduce their effectiveness in pest regulation (Madsen et al. 2004). However, a number of attributes afford them certain advantages over specialist natural enemies, namely their opportunistic habits allowing them to persist in the crop environment before pest species arrive and their predisposition to remain in place due to inherent risks associated with movement. These characteristics place generalist predators at a distinct advantage over specialists early in the season, potentially impacting pests during colonization. However, understanding the early-season population dynamics of predators in relation of alternative food sources is critical when making informed pest management decisions.

Spiders are particularly abundant in agroecosystems throughout the world, sometimes reaching densities in excess of 100 spiders/m$^2$ (e.g. Sunderland and Topping 1993). Despite their abundance, they have received relatively little attention, in contrast to many other predators, relative to their functional role in biological control. These voracious predators have rapid colonization abilities through the process of ballooning, allowing them to exploit transient field resources and immigrate into crop fields soon after planting (Weyman et al. 2002). Once in place, a number of micro-site and micro-climatic factors are responsible for maintaining a high density and diversity in such environments (Samu et al. 1999). However, spiders have traditionally been viewed as consummate insectivores (Nyffeler et al. 1994, Nyffeler, 1999), thus their conservation in agricultural landscapes has often focused on the supplementation of their diet through management approaches aimed at provisioning spiders with an increased availability of alternative prey. In recent years, it has become increasingly evident that these predators not only have the capacity to consume plant resources in agroecosystems, but the utilization of non-prey food positively affects fitness parameters (Taylor and Pfannenstiel 2009). Furthermore, their webs intercept very large quantities of plant resources in the field (Peterson et al. 2010), allowing spiders to utilize a readily available and nutritious food item as a supplement to prey items. This paper will examine the role of non-prey resources to agricultural spiders, quantifying the potential for dietary supplementation as a means to increase survival, growth and fecundity of these important natural enemies. Ultimately the role of integrative approaches that favor the promotion of a suite of natural enemies, that act in concert with one another in biological control, will be discussed, building on the recent body of evidence that suggests non-prey resources could be critical for the promotion of spider (and other) natural enemy populations in the field.

## References

Bilde, T., and S. Toft. 1997. Limited predation capacity by generalist arthropod predators on the cereal aphid, *Rhopalosiphum padi*. Biol. Agric. Hortic. 15: 143–150.

Hagen, K. S., and R. van den Bosch. 1968. Impact of pathogens, parasites, and predators on aphids. Ann. Rev. Entomol. 13: 325–384.

Madsen, M., S. Terkildsen, and S. Toft. 2004. Microcosm studies on control of aphids by generalist arthropod predators: effects of alternative prey. BioControl 49: 483–504.

Nyffeler, M. 1999. Prey selection of spiders in the field. J. Arachnol. 27: 317–324.

Nyffeler, M., W. L. Sterling, and D. A. Dean. 1994. Insectivorous activities of spiders in United States field crops. J. Appl.Entomol. 118: 113–128.

Peterson, J. A., S. A. Romero, and J. D. Harwood. 2010. Pollen interception by linyphiid spiders in a corn agroecosystem: implications for dietary diversification and risk-assessment. Arthropod-Plant Inte. 4: 207–217.

Samu, F., K. D. Sunderland, and C. Szinetár. 1999. Scale-dependent dispersal and distribution patterns of spiders in agricultural systems: a review. J. Arachnol. 27: 325–332.

Sunderland, K. D., and C.J. Topping. 1993. The spatial dynamics of linyphiid spiders in winter wheat. Memoirs of the Queensland Museum 33: 639–644.

Sunderland, K. D., J. A. Axelsen, K. Dromph, B. Freier, J.-L. Hemptinne, N. H. Holst, P. J. M. Mols, M. K. Peterson, W. Powell, P. Ruggle, H. Triltsch, and L. Winder. 1997. Pest control by a community of natural enemies. Act. Jutland. 72: 271–326.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Ann. Rev. Entomol. 47: 561–594.

Taylor, R. M., and R. S. Pfannenstiel. 2009. How dietary plant nectar affects the survival, growth and fecundity of a cursorial spider *Cheiracanthium inclusum* (Araneae: Miturgidae). Environ. Entomol. 38: 1379–1386.

Welch, K. D., R. S. Pfannenstiel, and J. D. Harwood. 2012. The role of generalist predators in terrestrial food webs: lessons for agricultural pest management. pp. 41–56. *In*: G. M. Gurr, S. D. Wratten, W. E. Snyder and D. M. Y. Read (eds.) Biodiversity and Insect Pests: Key Issues for Sustainable Management ), John Wiley & Sons Ltd., London, United Kingdom.

Weyman, G. S. K. D. Sunderland, and P. C. Jepson. 2002. A review of the evolution and mechanisms of ballooning by spiders inhabiting arable farmland. Ethol. Ecol. Evol. 14: 307–326.

# Consequences of facultative granivory to biological control in soil systems

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Seeds are a plentiful and nutritious form of food for many insects within a soil-dwelling community. The first step in understanding the granivore community associated with a seed species is diagnosing the degree to which arthropods within a habitat actually consume the seeds under natural conditions. We have coupled a protein-marking technique with ELISA-based gut content analysis to reveal the degree to which carabids and other arthropods consume protein-marked dandelion seeds under field conditions. Although frequently the most abundant animals trapped in pitfall samples, actual density samples and gut content analyses give a more realistic snapshot of where carabids fit within the communities associated with seeds or prey. Many of the facultative granivores in this system are also important predators of insect pests within the same habitat. Little attention has been given to how these insects balance their time and foraging efforts between hunting for seeds and prey. We investigated how carabid and crickets respond numerically to local seed rain, and through microscopic gut analysis examined how cricket diets changed in response to the availability of seeds. We found no changes in the insect community associated with high versus low seed inputs. One of the two species of crickets, *Gryllus pennsylvanicus* Burmeister (Orthopter: Gryllidae), altered its diet to consume more seeds when they were available, but this shift did not accompany diminished prey consumption in these crickets, leading us to believe that these insects may be food limited in the natural environment. New investigations in understanding the complex trophic interactions between carabids and seeds will likely make the use of granivory a more reliable source of pest management for weed species, as well as understanding the density and dispersion of many plant species within a habitat.

# Session 14: Generalist Predators for Plant Pest control: Applications, Prospects and Drawbacks

## The role of generalist predators in greenhouse crops

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There has been a long-standing debate whether biological control is promoted by specialist rather than generalist predators. Generalist predators are thought to be less suitable because of their interference with other natural enemies through intraguild predation or hyperpredation, switching behavior between prey and often relatively slow growth rate (van Lenteren and Woets 1988; Rosenheim et al. 1995; Chang and Kareiva 1999; Symondson et al. 2002). Moreover, exotic generalist predators are often considered as an environmental risk because of their potential effects on non-target species (van Lenteren et al. 2006). In contrast, specialist natural enemies are well-adapted to their prey and often show higher growth rates. Yet, biological control programs in greenhouse crops are increasingly based on releases of generalist predators and they appear to be successful. The first good results were obtained in sweet pepper where crop inoculations with generalist phytoseiid mites and anthocorid bugs successfully control thrips (Ramakers 2004). The selection of generalist predatory mites that are able to control whiteflies (Nomikou et al. 2002) has been a tremendous breakthrough in crops like cucumber and sweet pepper (Messelink et al. 2008; Calvo et al. 2009). As a result, biological control of whiteflies has shifted from using specialist parasitoids to using generalist predatory mites (such as *Amblyseius swirskii*) mainly. A recent success is the use of generalist mirid bugs in tomato for the control of the invasive pest *Tuta absoluta* (Lepidoptera: Gelechiidae) (Urbaneja et al. 2009). A key factor that explains these positive results is the ability of generalist predators to establish in crops prior to pest infestations by feeding on alternative food sources. This enables them to quickly detect and control pests that invade a crop, which benefits the resilience of cropping systems against pests.

Some greenhouse pests are still being tested for control mainly by specialized natural enemies. For example, the biological control of aphids, one of the remaining challenges in greenhouse crops, is based on specialist parasitoids, gall midges, coccinellidae or syrphidae, but these programs often fail (Bloemhard and Ramakers 2008). Crop inoculations with generalist predators may increase the control of aphids. Greenhouse trials show that the generalist predators *Orius majusculus* (Heteroptera: Anthocoridae) and *Macrolophus pygmaeus* (Heteroptera: Miridae) are promising candidates for this purpose. Here we discuss the complexities that occur when such predators are used in food webs of multiple pests and natural enemies through direct and indirect interactions among species (Messelink et al. 2012a). For example, generalist predatory mites, used for the control of thrips and whiteflies, strongly disrupt aphid control by the specialist gall midge *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) through hyperpredation of the eggs of this midge (Messelink et al. 2011). Thus, the benefits of these generalist predators must be weighed against the adverse effects on biological control by other natural enemies. The choice of using these predators will depend on several aspects, such as their importance for controlling pests, the possible alternative control measures and the potential risks of the pest species they prey on or favor by interference with other natural enemies. Other experiments show that the release of generalist predatory bugs together with specialist natural enemies of aphids increased aphid control, although the bugs can be potentially disruptive through intraguild predation on specialist aphid parasitoids and gall midges (Messelink et al. 2012b). Based on these and other results, we expect that the use of generalist predators in greenhouse crops will increase, but their potential negative effects on other natural enemies and associated environmental risks require thorough evaluations, including greenhouse experiments with multiple pests and natural enemies. Our future research will focus on selecting generalist predators that are adapted to certain crop plants and perform well on a mixture of pest species and other food sources present in these crops. Furthermore, we will continue developing techniques that allow the establishment of generalist predators, such as open rearing systems (Bennison et al. 2008), banker plants (Huang et al. 2011), habitat management (Perdikis et al. 2011) or food sprays (Wade et al. 2008). These developments may help in designing resilient cropping systems, based on the persistence of natural enemies, rather than cropping systems where natural enemies are applied as “biopesticides” in response to pest infestations.

## References

Bennison, J., K. Maulden, H. Maher, and M. Tomiczek. 2008. Development of a grower rearing-release system for *Atheta coriaria*, for low cost biological control of ground-dwelling pest life stages. IOBC/wprs Bulletin 32: 21–24.

Bloemhard, C. M. J. and P. M. J. Ramakers. 2008. Strategies for aphid control in organically grown sweet pepper in the Netherlands. IOBC/wprs 32: 25–28.

Calvo, F. J., K. Bolckmans, and J. E. Belda. 2009. Development of a biological control-based Integrated Pest Management method for *Bemisia tabaci* for protected sweet pepper crops. Entomol. Exp. App 133: 9–18.

Chang, G. C. and P. Kareiva. 1999. The case for indigenous generalists in biological control. pp. 103–115. *In* B. A. Hawkins and H. V. Cornell, (eds). Theoretical approaches to biological control. Cambridge University Press, Cambridge, U.K.

Huang, N. X., A. Enkegaard, L. S. Osborne, P. M. J. Ramakers, G. J. Messelink, J. Pijnakker, and G. Murphy. 2011. The banker plant method in biological control. Crit. Rev. Plant Sci. 30: 259–278.

Messelink, G. J., R. van Maanen, S. E. F. van Steenpaal, and A. Janssen. 2008. Biological control of thrips and whiteflies by a shared predator: Two pests are better than one. Biol. Control. 44: 372–379.

Messelink, G. J., C. M. J. Bloemhard, J. A. Cortes, M. W. Sabelis, and A. Janssen. 2011. Hyperpredation by generalist predatory mites disrupts biological control of aphids by the aphidophagous gall midge *Aphidoletes aphidimyza*. Biol. Control. 57: 246–252.

Messelink, G. J., M. W. Sabelis, and A. Janssen. 2012a. Generalist predators, food web complexities and biological pest control in greenhouse crops. pp. 191-214. *In* M. L. Larramendy and S. Soloneski, editors. Integrated pest management and pest control - current and future tactics. InTech, Rijeka.

Messelink, G. J., C. M. J. Bloemhard, M. W. Sabelis, and A. Janssen. 2012b. Biological control of aphids in the presence of thrips and their enemies. BioControl. DOI 10.1007/s10526-012-9462-2.

Nomikou, M., A. Janssen, R. Schraag, and M. W. Sabelis. 2002. Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. Exp. App. Acarol. 27: 57–68.

Perdikis, D., A. Fantinou, and D. Lykouressis. 2011. Enhancing pest control in annual crops by conservation of predatory Heteroptera. Biol. Control. 59: 13–21.

Ramakers, P. M. J. 2004. IPM program for sweet pepper. pp. 439–455. *In* K. M. Heinz, R. G. Van Driesche, and M. P. Parrella (eds). Biocontrol in protected culture. Ball Publishing, Batavia, Illinois.

Rosenheim, J. A., H. K. Kaya, L. E. Ehler, J. J. Marois, and B. A. Jaffee. 1995. Intraguild predation among biological control agents: theory and evidence. Biol. Control. 5: 303–335.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Ann. Rev. Entomol. 47: 561–594.

Urbaneja, A., H. Monton, and O. Molla. 2009. Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. J. app. Entomol. 133: 292–296.

van Lenteren, J. C., and J. Woets. 1988. Biological and integrated pest control in greenhouses. Ann. Rev. Entomol. 33: 239–269.

van Lenteren, J. C., J. Bale, E. Bigler, H. M. T. Hokkanen, and A. M. Loomans. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. Ann. Rev. Entomol. 51: 609–634.

Wade, M. R., M. P. Zalucki, S. D. Wratten, and K. A. Robinson. 2008. Conservation biological control of arthropods using artificial food sprays: Current status and future challenges. Biol. Control. 45: 185–199.

# Are generalist predators particularly suitable targets to be supported through non-prey food?

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The categorization of biological control agents as generalists or specialists has been strictly based on their prey/host range. In my presentation I will explore whether the level of specialism/generalism extends to the exploitation of non-prey food such as pollen or sugar sources. The potential and pitfalls of using non-prey food as a tool to enhance generalist predators will be discussed. Examples will be presented of how food supplements can be used to best realize the potential benefits and to avoid pitfalls.

# Tracking intraguild predation within arable crops using PCR and next generation sequencing

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Both communities and single species of generalist predators have been shown to be capable of limiting pest population growth (Symondson et al. 2002). However, a complex suite of factors determine whether they are effective at doing so, including diversity and abundance of both predators and prey, predator traits, availability of alternative prey, prey preferences, food web dynamics and potentially negative interactions such as intraguild predation. Strategies for addressing such complexity include attempts to increase biodiversity and predator abundance within arable ecosystems through habitat manipulation and other IPM measures. However, this 'black box' approach does not always work.

Recent advances in molecular diagnostics have made it possible for the first time to track the complete diets of generalist predators, with the prospect of constructing realistic food webs based directly upon trophic links measured in the field rather than upon extrapolation from laboratory studies. Here we report field experiments demonstrating extensive intraguild predation in cereal fields. Specifically we present data showing predation by a range of beetle and spider predators upon parasitoids and parasitized hosts, predation by carabid beetles on spiders and spider-spider interactions. We demonstrate how next generation sequencing can be used to analyse the complete diets of generalist vertebrate and invertebrate predators that feed on pests, alternative prey and intraguild competitors. Such information should allow us to design more precisely IPM strategies that will lead to pest suppression by native natural enemy communities.

## Predation on parasitized hosts

A potentially significant form of intraguild predation is where predators feed on parasitized hosts. We used multiplex PCR (Harper et al. 2005) to analyse the gut contents of beetles and spiders in winter wheat, using primers developed against a complete community of parasitoids and hyperparasitoids attacking cereal aphids (Traugott et al. 2008). From this we constructed a bipartite food web (Figure 1) showing predation by each species or predator group, with the initial null hypothesis that if an individual predator tested positive for both aphid and parasitoid DNA then the most likely explanation was predation on a parasitized aphid (Traugott et al. 2012).

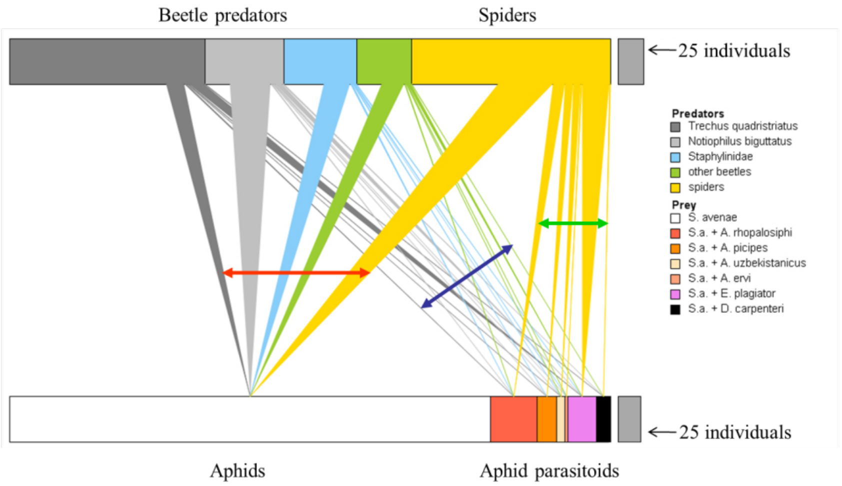

Figure 1. Trophic links between predators, aphids and parasitized aphids, determined using multiplex PCR. The gut contents of the predators were analysed using aphid and parasitoid-specific primers. The coloured arrows are discussed in the main text.

There were strong links in the wheat crop between all of the predators and aphids (red arrow). There were weak links between the beetles and the parasitized aphids (blue arrow), suggesting that parasitized aphids were eaten in approximately the proportions found in the field. The links between spiders and parasitoids, however, were stronger (green arrow). Although this could be interpreted as disproportional predation on parasitized hosts it is more likely that the spiders (unlike the beetles) are catching adult parasitoid (in their webs) then eating aphids and parasitoids as two separate meals. This study illustrates the power to construct food webs from PCR-based data, but also the problem with subsequent interpretation.

## Carabid-spider interactions

Both carabid beetles and linyphiid spiders have been shown to be major predators of aphids and other pests in wheat fields. However, unidirectional intraguild predation by the carabids, *Pterostichus melanarius* (Coleoptera: Carabidae), on spiders (Araneae: Tetragnathidae and Linyphiidae), could potentially reduce the impact that these generalist predators may have on aphid numbers. Primers were designed for the four most common species of spider (encompassing ca. 85% of the spider community) at our field site (Davey et al. 2012). Multiplex PCR was used to determine rates of spider consumption by the beetles. Beetle and spider densities in the field were recorded with juvenile spiders identified using species-specific primers. A Monte Carlo simulation approach was used to compare observed rates of predation with expected rates, based upon a random, no-choice model (Figure 2). Rates of intraguild predation were high, with 44% of beetles testing positive for spider DNA in June. The most common species of spider, *Tenuiphantes tenuis* (Araneae: Linyphiidae), was taken disproportionally frequently, showing evidence of 'switching' (Murdoch 1969) and/or strong prey choice for a species found close to the ground. A species found further up the plants, *Bathyphantes gracilis* (Araneae: Linyphiidae), was taken less frequently than expected from a random model, supporting the hypothesis that accessibility, and hence encounter rates, was driving prey choice. Interestingly, high rates of predation on *T. tenuis* did not prevent strong growth in the numbers of this predator through the summer months, suggesting that encouraging high densities of both beetles and spiders is in practice a valid strategy for enhancing aphid control (Davey et al. 2012).

## Spider-spider interactions and use of Next Generation Sequencing (NGS)

Current unpublished work on spider-spider interactions in winter wheat, over a two-year period using prey-specific primers, has revealed extensive intraguild predation within a mixed spider community (results will be reported elsewhere). However, although the multiplexing of primers allows to screen for predation on a range of species simultaneously, there is always a need to predict which species are likely to be consumed, so that primers can be developed. This may not be adequate in many ecosystems, especially where biodiversity is high (such as in flower-rich field boundaries or tropical agroecosystems). Recently, NGS has been used to analyse the diets of vertebrates and herbivores and examples of the former from our laboratory will be illustrated in the talk. No one has until now used this technology to analyse predation by invertebrates in the field, looking at predation on pests, non-pest alternative prey and intraguild predation simultaneously. For one species of spider, *Oedothorax fuscus* (Araneae: Linyphiidae), we conducted next generation sequencing on a pooled sample of spiders to examine their complete dietary range (Pinol et al. submitted). For spiders inhabiting the boundary of a field of winter barley the largest number of sequences were for Collembola, from four families, followed by Lepidoptera, aphids and other spiders (intraguild predation). The dominance of Collembola sequences in the guts of linyphiid spiders, such as these, confirmed results obtained earlier using specific primers (e.g. Agustí et al. 2003).

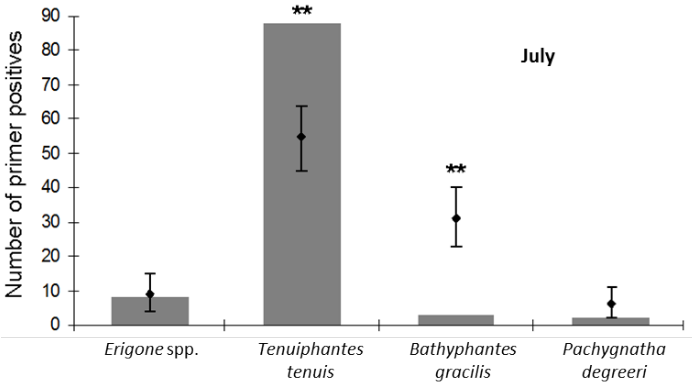

Figure 2. Monte Carlo simulations, comparing the observed numbers of carabids testing positive for spider prey (grey bars) with the range of values enclosing 95% of the simulated predation rates (vertical bars). ** $P < 0.01$. Modified from Davey et al. (2012).

## Conclusion

Intraguild predation is clearly widespread in agricultural fields (see also Gagnon et al. 2011). Equally important are trophic interactions involving predators consuming other predators that do not share the same prey, for example spiders eating Collembola (Agustí et al. 2003) that are themselves predators of beneficial nematodes (Read et al. 2006). These interactions need to be fully integrated within food web analyses that attempt to predict beneficial outcomes (pest regulation). Intraguild predation can in theory have both stabilising and destabilising effects on food webs, under different circumstances (McCann and Hastings 1997; Holt and Polis 1997). Molecular diagnostics have shown that such interactions can now be tracked directly in the open f field to measure outcomes within agroecosystems.

## Acknowledgements

Funding for the work reported here came from a variety of sources, but principally from the Natural Environment Research Council (UK), the European Union (Marie Curie Fellowship), the Spanish Government (personal fellowship) and the University of Barcelona. Other people involved with this work in various ways included J. R. Bell, D. A. Bohan, M.W Bruford, J. M. Holland, R. A. King, G. Mir, R. Reader, L. Raso and D. Sint. We thank John Wiley Ltd. for permission to reprint Figure 2 (modified from Figure 3 in Davey et al. 2012).

## References

Agustí, N., S. Shayler, J. D. Harwood, I. P. Vaughan, K. D. Sunderland, and W. O. C. Symondson. 2003. Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. Mol. Ecol. 12: 3467–3475.

Davey, J. S., I. P. Vaughan, R. A. King, J. R. Bell, D. A. Bohan, M. W. Bruford, J. M. Holland, and W. O. C. Symondson. 2012. Intraguild predation in winter wheat: prey choice by a common epigeal carabid consuming spiders. J. Appl. Ecol.. DOI: 10.1111/1365-2664.12008.

Gagnon A. E., G. E. Heimpel, and J. Brodeur. 2011. The ubiquity of intraguild predation among predatory arthropods. PLoS ONE 6: e28061.

Harper, G. L., R. A. King, C. S. Dodd, J. D. Harwood, D. M. Glen, M. W. Bruford, and W. O. C. Symondson. 2005. Rapid screening of invertebrate predators for multiple prey DNA targets. Mol. Ecol. 14: 819–827.

Holt, R. D., and G. A. Polis. 1997. A theoretical framework for intraguild predation. Am. Nat. 149: 745–764.

McCann, K., and A. Hastings. 1997. Re-evaluating the ominivory-stability relationship in food webs. Proc. Roy. Soc. B – Biol. Sci. 264: 1249–1254.

Murdoch, W. W. 1969. Switching in general predators. Experiments on predator specificity and stability of prey populations. Ecol. Monogr. 39: 335–354.

Piñol, J, V. San Andrés, E. L. Clare, G. Mir, and W. O. C. Symondson. (Submitted). Analysis of the invertebrate diets of generalist predators (the Linyphiid spider *Oedothorax fuscus*) using Ion Torrent sequencing. Mol. Ecol. Res.

Read, D. S., S. K. Sheppard, M. W. Bruford, D. M. Glen, and W. O. C. Symondson. 2006. Molecular detection of predation by soil micro-arthropods on nematodes. Mol. Ecol. 15:1963–1972.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. (2002). Can generalist predators be effective biocontrol agents? Ann. Rev. Entomol. 47: 561–594

Traugott, M., J. R. Bell, G. R. Broad, W. Powell, F. J. F. van Veen, I. M. G. Vollhardt, and W. O. C. Symondson WOC. 2008. Endoparasitism in cereal aphids: molecular analysis of a whole parasitoid community. Mol. Ecol. 17: 3928–3938.

Traugott, M., J. R. Bell, L. Raso, D. Sint, and W. O. C. Symondson. 2012. Generalist predators disrupt parasitoid aphid control by direct and coincidental intraguild predation. Bull. Entomol. Res. 102: 239–247.

# Predator diversity, weed abundance and biological control: do generalist predators enhance or inhibit the impact of specialists?

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In the context of drastic biodiversity losses due to the expansion of modern, chemically intensive agriculture (Stoate et al. 2001; Robinson and Sutherland, 2002), organic agriculture shows promises for biodiversity conservation (Bengtsson et al. 2005). It is still unclear to what extent biological pest control benefits from biodiversity (Straub et al. 2008) and, if so, what mechanisms are involved. In many annual crops generalist predators are important pest regulators early in the crop-season when specialists may be uncommon (Ekbom and Wiktelius, 1985; Symondson et al. 2002). The role of generalists later in the crop-cycle, when they can interact with specialist predators that have colonized the field, remains less clear. The presence of generalist predators may have negative or positive effects on biological pest control depending on the relative importance of processes such as intraguild predation, resource complementarity, and facilitation (Straub et al. 2008). In addition, the presence of weeds, a distinguishable characteristic in organic fields, might moderate or interfere with the mechanisms involved by adding structural complexity and/or additional resources (Janssen et al. 2007; Diehl et al. 2011). Further understanding of such mechanisms are now made possible with the development and constant improvement of DNA-based molecular gut-content analysis to study interactions in terrestrial predator-prey systems (King et al. 2008, 2011).

We will present results from a field cage experiment where we manipulated the diversity of generalist and specialist predators, and weed abundance to study their impact on biological control of cereal aphids. This experiment was carried out in an organic barley field around Uppsala, Sweden, in 2x2x2m cages in July 2012. We used a substitutive, cross design where we tested the effect of the presence/absence of weeds and the functional richness of predators on cereal aphid population development. Prior to the start of the experiment: 1) the natural weed community was transplanted or weeded according to treatment, 2) naturally occurring predators were removed using both pitfall traps and manual search; and 3) cereal aphids (*Rhopalosiphum padi* and *Sitobion avenae*) were inoculated to the cages and their densities monitored to reach medium to high infestation level. Predator treatments consisted of generalist predators (*Pterosticus spp.)* and specialists (4-7 day-old larvae of *Coccinela septempunctata* and *Chrysoperla carnea*) introduced as single species or multiple species. We recorded aphid and non-pest prey population abundances and sampled predators every 4 days for a 12 day period, using low disturbance sampling methods. Predators collected were immediately frozen in dry ice in the field and stored at -80 °C before identification to species level, and molecular analysis.

A DNA-based approach using multiplex PCR currently in development will provide information on predators' gut contents, which will allow analysis of the frequency of intraguild, aphid and non-pest prey predation in different treatments. We will test the following hypotheses: 1) the structural complexity and/or additional resources from weeds change the structure of food webs, which results in an increase of aphid predation; 2) an increase of functional richness of predators result in an increase of aphid biological control due to predator complementation and/or facilitation and 3) functionally rich predator communities benefit from weeds that decrease intraguild predation, and increase biological control.

## References

Bengtsson, J., J. Ahnström, and A.- C.Weibull. 2005. The effects of organic agriculture on biodiversity and abundance: a meta-analysis. J. App. Ecol. 42: 261–269.

Diehl, E., V. Wolters, and K. Birkhofer. 2011. Arable weeds in organically managed wheat fields foster carabid beetles by resource- and structure-mediated effects. Arthropod-Plant Interactions 6: 75–82.

Ekbom, B. S., and S. Wiktelius. 1985. Polyphagous arthropod predators in cereal crops in central Sweden, 1979-1982. Z. Ange. Entomol. 99: 433–442.

Janssen, A., M. W. Sabelis, S. Magalhães, M. Montserrat, and T. Van der Hammen. 2007. Habitat structure affects intraguild predation. Ecology 88: 2713–2719.

King, R. A., D. S. Read, M. Traugott, and W. O. C Symondson. 2008. Invited review: Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol. Ecol. 17: 947–963.

King, R. A., R. Moreno-Ripoll, N. Agustí, S. P. Shayler, J. R. Bell, D. A. Bohan, and W. O. C Symondson. 2011. Multiplex reactions for the molecular detection of predation on pest and nonpest invertebrates in agroecosystems. Mol. Ecol. Res. 11: 370–373.

Robinson, R. A., and W. J. Sutherland. 2002. Post-war changes in arable farming and biodiversity in Great Britain. J. App. Ecol. 39: 157–176.

Stoate, C., N. Boatman, R. Borralho, C. R. Carvalho, G. R. D. Snoo, and P. Eden. 2001. Ecological impacts of arable intensification in Europe. J. Environ. Man. 63: 337–365.

Straub, C. S., D. L. Finke, and W. E. Snyder. 2008. Are the conservation of natural enemy biodiversity and biological control compatible goals? Biol. Control 45: 225–237.

Symondson, W.O.C., K. D. Sunderland, and M.H. Greenstone. 2002. Can generalist predator be effective biocontrol agent? Ann. Rev. Entomol. 47: 561–594.

# Unravelling non-pest prey feeding by generalist predators using molecular tools and its implications for conservation biological control

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Generalist predators such as carabid beetles and spiders have been shown to feed on a large variety of agricultural pests, thus providing an important contribution to keep pest densities below economic damage thresholds. The provision of this ecosystem service can translate into a reduction of pest-inflicted yield losses comparable to crop protection by insecticide use (Östman et al. 2003). Generalist predators, however, do not only feed on pests, but may also prey on potential competitors which may attenuate the overall pest suppression effect. Moreover, these predators are known to consume non-pest prey such as collembolans, dipterans and earthworms. This feeding on alternative, non-pest prey plays an important and contradictory role regarding the efficacy of pest control delivered by these natural enemies (Snyder and Tylianakis 2012): alternative prey may, on one side, benefit pest control by supporting generalist predators' populations when the pest is unavailable (von Berg et al. 2009). It may also reduce intraguild trophic interactions between natural enemies, a mechanism that has received less attention so far. However, on the other side, non pest foods may cause predators to switch away from feeding on the pest and reduce the efficacy of biological control (Halaj and Wise 2002). Moreover, non pest foods might also attract intraguild predators and diminish the control exerted by key natural enemies (Jonsson et al. 2009).

Most empirical evidence of these trophic interactions stems either from manipulative experiments where trophic connections are indirectly inferred or from studies where the assessment of feeding interactions is limited to a few taxa. This is mainly due to the practical difficulties inherent in identifying and quantifying complex predator-prey interactions in the field, especially when species with a wide prey spectrum are involved (Symondson 2012). Novel molecular techniques in trophic relationship research facilitate the investigation of these feeding links by detecting and identifying prey DNA within the gut content of generalist predators (King et al. 2008; Pompanon et al. 2012). Nowadays, techniques such as multiplex PCR and high-throughput applications for visualizing PCR products allow screening of consumers' gut contents for several food sources within one assay (Sint et al. 2012), greatly facilitating the assessment of the feeding behavior of generalist predators in multiple-species systems.

In this talk we will demonstrate how DNA-based approaches to prey detection allow tracking of non-pest feeding by generalist predators. Using case studies in cereal systems, the role of non-pest prey will be examined. In the first study, the feeding choices of the larvae of cantharid and carabid beetles were examined. The adults of these beetles are important antagonists of pests during the cropping season and their population densities are affected by larval survival. Knowledge of the type of prey sustaining the juvenile predators is therefore crucial for improving their survival. As many of these species accomplish their larval development during the cold season, this study was focusing on an invertebrate community present in an Austrian arable field and grassland site during autumn, winter and early spring (Eitzinger and Traugott 2011). The invertebrate community was dominated by earthworms and collembolans as well as by predatory beetle larvae and linyphiid spiders. As the immature beetles are fluid feeders, which impedes a microscopic analysis of their gut content, multiplex PCR assays were developed to examine the feeding on detritivores and intraguild prey, i.e. larval beetles and linyphiid spiders. Over 500 beetle larvae which were collected throughout the cold season were screened for prey DNA: more than 60% tested positive for earthworm and collembolan DNA while only a few individuals (0.2-1%) contained DNA of predatory taxa. Our findings demonstrate that that the cold-adapted juvenile predators are strongly linked to the detrital food chain. Consequently, by improving the habitat conditions for detritivores year round will retain and sustain predatory beetles during their whole life cycle in arable land and foster their ability to control pests.

Currently we are extending this work to generalist predators which are active during the vegetation period to unravel which role non-pest prey plays for adult carabid beetles and spiders. Within this work we will explore how farming system and landscape complexity affect the food web interactions. Based on the outcomes of this work, the functional implications of non-pest prey feeding for conservational biological control will be discussed and the future prospects for generalist predators for plant pest control identified.

## References

Eitzinger, B., and M. Traugott. 2011. Which prey sustains cold-adapted invertebrate generalist predators in arable land? Examining prey choices by molecular gut content analysis. J. Appl. Ecol. 48: 591–599.

Halaj, J., and D. H. Wise. 2002. Impact of a detrital subsidy on trophic cascades in a terrestrial grazing food web. Ecology 83: 3141–3151.

Jonsson, M., S. D Wratten, K. A. Robsinson, and S. A. Sam. 2009. The impact of floral resources and omnivory on a four trophic level food web. Bull. Entomol.Res. 99: 275–285.

King, R. A., D. S. Read, M. Traugott, and W. O. C. Symondson. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Mol. Ecol. 17: 947–963.

Östman, Ö., B. Ekbom, and J. Bengtsson. 2003. Yield increase attributable to aphid predation by ground-living polyphagous natural enemies in spring barley in Sweden. Ecol. Econ. 45: 149–158.

Pompanon, F., B. E. Deagle, W. O. C. Symondson, D. S. Brown, S. N. Jarman, and P. Taberlet. 2012. Who is eating what: diet assessment using next generation sequencing. Mol. Ecol. 21: 1931–1950.

Sint D., L. Raso, and M. Traugott. 2012. Advances in multiplex PCR: balancing primer efficiencies and improving detection success. Meth. Ecol. Evol. 3: 898–905.

Snyder, W. E., and J. M. Tylianakis. 2012. The Ecology of biodiversity-biocontrol relationships, pp. 24–40. *In* G.M. Gurr, S.D. Wratten, W.E. Snyder, and D.M.Y, Read (eds), Biodiversity and Insect pests: Key issues for sustainable management. Wiley and Sons, New Tork, USA.

Symondson, W. O. C. 2012. The molecular revolution: using plolymerase chain ractionbased methods to explore the role of predators in terrestrial food webs. pp. 166–184. *In* G.M. Gurr, S.D. Wratten, W.E. Snyder, and D.M.Y, Read (eds), Biodiversity and Insect pests: Key issues for sustainable management. Wiley and Sons, New Tork, USA.

von Berg, K., C. Thies, T. Tscharntke, and S. Scheu. 2009. Cereal aphid control by generalist predators in presence of below ground alternative prey: Complementary predation as affected by prey density. Pedobiologia 53: 41–48.

# Session 15: Consequences of Reduced-Impact Pesticides for Agents of Biological Control and Top-Down Suppression of Arthropods in Managed Landscapes

## The influence of crop management tactics, including seed-applied insecticides, on biological control of slugs in no-till crop fields

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Slugs (Mollusca: Gastropoda: Agriolimacidae: *Deroceras* and Arionidae: *Arion* spp.) are destructive pests of myriad field crop species during crop establishment in reduced-tillage settings, particularly in Pennsylvania and other parts of the mid-Atlantic and Northeastern United States. Current management options for slugs are limited and farmers in the region have expressed a desire for research on alternatives for improved slug control. Unfortunately, development of alternative management strategies for slugs is limited by our poor understanding of basic aspects of slug ecology in the region. Our research uses laboratory and field experiments to provide insight on slug ecology, natural history, trophic interactions, and management.

To better understand the potential for biological control to contribute to slug management programs in Pennsylvania, we use laboratory experiments to explore predation on slugs by common generalist arthropod predator species. Despite literature claims of their preferences for slugs, wolf spiders (Araneae: Lycosidae) and cantharid (Coleoptera: Cantharidae) larvae were disinclined to eat slugs; however, consistent with previous research two common ground beetle species from Pennsylvania crop fields, *Chlaenius tricolor* Dejean and *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae), preyed on slugs and protected soybean seedlings from damage, indicating a potentially important role for them in biological control.

Next, we examined how crop management decisions influence slugs, insect pests, and predators in reduced-tillage maize systems, in field studies spanning two years. Weed (reduced-herbicide or standard no-till, herbicide-based management) and manure management (broadcast or inject manure applications) tactics had few effects on pest activity, crop damage, or predator activity in either year, though cultivation reduced late-season slug activity in 2011. Low-external-input crop rotations using cover crops were somewhat more vulnerable to slugs compared to a control rotation using pre-emptive insect management that included broadcast application of pyrethroid insecticides. Furthermore, in the second year of our study, the low-external-input rotations supported higher levels of predation on sentinel caterpillars, and comparable or lower levels of slug activity late in the season compared to the higher-input, insecticide-reliant, control rotation. Overall the low-external-input rotations supported higher levels of biological control and its crop productivity was competitive with that of the more conventional, pre-emptive pest management strategy.

Finally, we examined the influence of the common seed treatment, thiamethoxam, on slug damage to soybeans in a tritrophic context. In laboratory experiments, consistent with previous research, thiamethoxam did not significantly influence slug survival or feeding behavior on soybean. However, slugs that were fed for one week on soybeans grown from seeds treated with thiamethoxam were poisonous to the ground beetle, *Chlaenius tricolor*. Our results suggest that neonicotinoid seed treatments do not prevent slug damage to soybean, and in fact may exacerbate slug damage if slugs pass these insecticides to their predators. This possibility is currently being evaluated in field studies.

In summary, our work shows that arthropod predators commonly found in Pennsylvania crop fields hold strong potential to contribute to natural control of slug populations. Moreover, use of an integrated pest management-based pest management strategy that avoids unnecessary applications of broadcast insecticide can provide competitive crop yields while contributing to development of predator populations that have an improved chance of contributing to pest control. Lastly, chemical management tactics that are typically initiated outside an integrated pest management framework, like seed-applied insecticides, appear to have potential to disrupt biological control. Therefore, pest management and biological control researchers need to consider the entire suite of crop management strategies used in agricultural systems to determine which can foster natural enemy populations and biological control as well as those that can disrupt these valuable ecosystem services.

# Impact of pesticides on phytophagous and predatory mites: case studies in annual and perennial crop systems

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Predator-prey interactions in crops may be affected by pesticides used to control various pests or pathogens and thus knowledge of pesticide side-effects is essential for Integrated Pest Management (IPM). Biological control strategies based on predatory mites can maintain spider mites populations below economic injury levels but the use of pesticides detrimental to predators can induce spider mite outbreaks. Studies evaluating the effects of pesticides on both predator and prey can predict their impact on mite communities. The outcome of a pesticide application on mite communities in real applicative situations can be influenced by factors in addition to the pesticide's intrinsic toxicity. The effect of pesticides on the relationships between predatory mites and spider mites were studied in some annual and perennial crop systems. The influence of the routes of exposure to pesticides and that of alternative foods availability on the impact of pesticides was investigated.

In annual crops *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) is a major pest that can be kept under control by the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Mesostigmata: Phytoseiidae). These mites are naturally occurring in the Mediterranean area. Lethal and sub-lethal effects of botanical and reduced-risk insecticides recommended for the control of aphids, whiteflies and thrips were evaluated on *T. urticae* and *P. persimilis* females (Duso et al. 2008). Pyrethrins and rotenone were more toxic to *P. persimilis* than to *T. urticae*; azadirachtin, *Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae) and pymetrozine showed an opposed tendency. Imidacloprid had a comparable impact on predator and prey. *Beauveria bassiana* was the most effective on *T. urticae* eggs, while rotenone significantly reduced *P. persimilis* egg hatching. Mites can be exposed to pesticides through different routes of exposure: direct exposure (topical) to pesticide droplets, exposure to pesticide residues and exposure to contaminated food sources. For systemic pesticides such as neonicotinoids, the exposure to systemically contaminated food should be considered. In another experiment the effects of four pesticides (thiamethoxam, *B. bassiana*, pyrethrins and spinosad) were assessed on *T. urticae* and *P. persimilis* thought different routes of exposure. Thiamethoxam was toxic to *T. urticae* and *P. persimilis*. Systemic exposure was more detrimental for *T. urticae* than for *P. persimilis* (Pozzebon et al. 2011). Spinosad reduced survival and fecundity of both *T. urticae* and *P. persimilis* resulting in greater toxicity to the prey than to the predator. *Beauveria bassiana* showed significant effects on the survival, fecundity and egg hatch of spider mites, and was more toxic to the spider mite than to its predator. Pyrethrins were moderately toxic to *T. urticae* but highly toxic to *P. persimilis*. The effect of pesticides was influenced by routes of exposure with some variations among different compounds. Considering different routes of exposure in toxicological assays can help in understanding interactions among pesticides, pests and beneficial mites. Likelihood of success for IPM is increased by adopting agricultural practices that reduce pesticide routes of exposure for predators (i.e. root application or predator releases on plants with aged residues).

Spider mites can be important pests in apple orchards and vineyards. Generalist predatory mites like *Amblyseius andersoni* (Chant) and *Kampimodromus aberrans*

(Oudemans) (Mesostigmata: Phytoseiidae) play an important role as  agents in these perennial crop systems. These predatory mites are characterized by a wide food range that includes spider mites, pollen, fungi and other food sources. Field and laboratory experiments were performed to evaluate the effects of pesticides commonly used in perennial crop systems on predatory mites and spider mites. On apple, the effects of some insecticides (e.g., neonicotinoids, pyrethroids and IGRs) were evaluated on *A. andersoni, K. aberrans* and their prey *Panonychus ulmi* (Koch) (Trombidiformes: Tetranychidae). Lethal and sub-lethal effects of the same products were evaluated on *A. andersoni, K. aberrans, P. ulmi* and *T. urticae* in the laboratory. Results from apple experiments evidenced that pyrethroids were the more toxic insecticides to predatory mites and that induced mite pest outbreaks. Field effects of neonicotinoids were partly explained by laboratory trials. Neonicotinoids decreased predatory mite fecundity and some of them increased mite escaping. Contrasting results regarding neonicotinoids on spider mites were observed among different seasons and between field and laboratory experiments.

Pollen is an alternative food source for generalist predatory mites. The effect of its availability on the impact of pesticides on *K. aberrans* was evaluated in semi-field and laboratory conditions. Fresh pollen availability can mitigate the negative effect of pesticides favoring resilience in predatory mite populations (Duso et al. 2011).

On grapevine the effect of four pesticides (etofenprox, thiamethoxam, chlorpyrifos and *B. bassiana*) was evaluated on *K. aberrans* and its prey *Eotetranychus carpini* (Oudemans) (Trombidiformes: Tetranychidae). Etofenprox was detrimental to predators and induced a spider mite increase. The prey/predator ratios were similar in the remaining treatments.

## References

Duso, C., V. Malagnini, A. Pozzebon, M. Castagnoli, M. Liguori, and S. Simoni. 2008. Comparative toxicity of botanical and reduced-risk insecticides to Mediterranean populations of *Tetranychus urticae* and *Phytoseiulus persimilis* (Acari Tetranychidae, Phytoseiidae). Biol. Control 47: 16–21.

Pozzebon, A., C. Duso, P. Tirello, and P. B. Ortiz. 2011. Toxicity of thiamethoxam to *Tetranychus urticae* Koch and *Phytoseiulus persimilis* Athias-Henriot (Acari Tetranychidae, Phytoseiidae) through different routes of exposure. Pest Manag. Sci. 67: 352–359.

Duso, C., A. Pozzebon, M. Baldessari, V. Girolami, G. Angeli, P. Tirello, M. Lorenzon, V. Malagnini, and G. Pellizzari. 2011. Availability of alternative foods can influence the impact of pesticides on predatory mites (Acari): a summary of the evidence, pp. 124–130. *In* G. J. de Moraes and  H. Proctor (eds.),  Acarology XIII: Proceedings of the International Congress, 23-27 August 2010, Recife-PE, Brazil. Zoosymposia 6. Magnolia Press, Auckland, New Zealand.

# Soybean aphid resistant seed treatments and genetics: Effects on biological control

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The relatively recent invasion of soybean aphids (SBA), *Aphis glycines* Matsumura (Hemiptera: Aphididae), in U.S. soybean crops has resulted in pressure to develop effective means of management. Infestations are sporadic; treatment is often unnecessary some years. Past research has indicated that SBA populations can be reduced below economically damaging levels by coccinellids and *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). In Indiana, *Orius* is a key predator of SBA, capable of suppressing colonizing populations (Rutledge et al. 2004, Rutledge and O'Neil 2005). *Orius* relies on thrips (Thysanoptera: Thripidae) populations early in the season, and a reduction of thrips could result in season long effects on the resident *Orius* population. It is possible that if thrips populations are greatly reduced, *Orius* populations may fail to build prior to aphid arrival. Despite its utility, biological control is rarely employed as the sole management technique.

The more widely used system utilizes a monitoring and treatment threshold of 250 aphids/plant (Ragsdale et al. 2007). Although effective, the required scouting can be time-consuming, costly, and inconvenient. Because of this, many producers have welcomed a recently developed option of in-plant protection from this pest and others. In-plant protection is achieved via neonicotinoid seed treatments and SBA resistant genetics. The use of seed treatments provides systemic pest control against a variety of above and below-ground insect pests, including the SBA. These products are touted as being in line with integrated pest management ideals. Advertisements suggest a potential side benefit of seed treatments are reduced effects on beneficial insects (Syngenta 2010) compared to conventional treatments of foliar insecticides applied at times of infestation.

These claims disregard the omnivorous nature an ever increasing number of beneficial insects. Our research is aimed at determining potential non-target effects of neonicotinoid seed treatments in soybeans. We hypothesized that 1) *Orius* populations will be reduced via exposure to toxins from plant fluid and pollen feeding, and 2) *Orius* populations will be reduced as a result of prey reduction.

We examined the effects of a neonicotinoid seed treatment on insect populations over two years of field experiments. Thrips and aphid numbers were unaffected by the seed treatments, and further laboratory analyses found that insecticide concentrations in foliage were below detectable limits 30 days post-planting. Laboratory feeding assays examined adult *Orius* mortality when limited to pollen and anthers collected from neonicotinoid, fungicide, or untreated seed. The trials revealed no differences in mortality across treatments and were supported by no detectable levels of neonicotinoids in the pollen and anthers. Lastly, no differences in yield were observed.

These results provide little evidence to benefits gained by producers using neonicotinoid seed treatments. They do however provide supporting information to the discussion of non-target effects of reduced risk insecticidal treatments and beneficial arthropods.

## References

Ragsdale, D.W., B.P. McCornack, R.C. Venette, B.D. Potter, I.V. Macrae, E.W. Hodgson, M.E. O'Neal, K.D. Johnson, R. J. O'Neil, C.D. Difonzo, T.E. Hunt, P.A.

Glogoza, and E.M. Cullen. 2007. Economic Threshold for Soybean Aphid (Hemiptera: Aphididae). J. Econ. Entomol. 100: 1258-1267.

Rutledge, C.E., R.J. O'Neil, T.B. Fox, and D.A. Landis. 2004. Soybean aphid predators and their use in integrated pest management. Ann. Entomol. Soc. Am. 97: 240-248.

Rutledge, C.E., and R. J. O'Neil. 2005. *Orius insidiosus* (Say) as a predator of the soybean aphid *Aphis glycines* Matsumura. Biol. Control 33: 56-64.

Unknown Affiliate, Syngenta Corporation. Published March, 2010. Soybean Aphid Management System. Complete URL. http://www.syngenta.com/country/us/en/Seeds/PDF%20and%20Other%20document%20Storage%20Library/Ag_Reports/AMS_Syngenta_FINAL%20.pdf. Accessed February 13, 2011.

# Biological control and pesticide disturbance: integrating life history data into predictive population models

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Parasitoid wasps are important control agents for a suite of economically important arthropod pests. However, it is well known that Hymenoptera in general are highly vulnerable to the effects of chemical pesticides, and so represent an opportunity to better understand the compatibility of biological and chemical control of pests. Closely related species are often used in risk assessment trail in order to better assess the effects of different chemical toxicants on hymenoptera. Most of these tests focus on acute toxicity, with few exploring longer-term population outcomes. Here, using a simple mathematical framework, we quantify the relative vulnerability of a suite of parasitoid species to both acute and sublethal effects of a toxicant acting on fecundity and survivorship. Using an equation that describes a critical extinction threshold, we calculate levels of reduction in fecundity and survivorship above which parasitoid species important for biological control are driven to local extinction. We apply this framework to four economically important braconid biological control agents and find that threshold levels vary widely among them. Furthermore, we use sensitivity analysis to characterize the relationship between life history traits and population response to pesticide disturbance for each species studied. Our findings imply that caution should be exercised in assessing how pesticides may affect assemblages of species important for biological control.

Parasitoid wasps are famously effective biological control agents, adept at finding, tracking, and regulating host populations, and are the primary control agents for many economically important crops pests (Godfray 1994, Murdoch 1994, Casas 2000). However, parasitoid populations can also be quite susceptible to the effects of chemical pesticides (Theiling and Croft 1998). Because most integrated pest management regimes involve the use of some level of chemical pesticides, the compatibility of parasitoid and chemical control has been the subject of many lab and field studies (Nowak et al. 2001, Jepsen et al. 2007, Stark et al. 2007, Carmo et al. 2010). In many cases, several parasitoid species may be found in an agroecosystem, and they may have different population responses to pesticide sprays. Using a simple mathematical model parameterized with life history data, we explore here the variable population responses of several different parasitoid species subjected to pesticide disturbance.

Traditionally, the effects of pesticides and other toxicants have been explored by conducting closed, controlled laboratory studies on organisms that have been dosed with successively higher amounts of a toxicant until 50% of the population has been killed. This approach, known as “$LD_{50}$” or “$LC_{50}$” studies (depending on whether one is measuring the amount in the target study organism or in an environmental medium such as water, respectively), has been critically challenged in the past decade in a series of data-rich modeling studies (Wennergren and Stark 2000, Calow et al. 2001, Stark and Banks 2003, Stark et al. 2004). More sophisticated approaches incorporating life history data (such as survival or reproductive output) into simple mathematical (e.g., Leslie matrix) models have led to improvements in assessing the risk of toxicants to species of concern, by focusing on *population outcomes* rather than static toxicity tests

(Banks and Stark 1998, Calow et al. 2001, Stark and Banks 2003, Lopes et al. 2007, Banks et al. 2008, Forbes & Calow 2008).

In the current study, we used a standard Leslie matrix model (Leslie 1945) to represent the population growth through time of a stage-structured population with four life stages (neonates, juveniles, young reproductives, and mature reproductives). The number of individuals in each of these four stage classes is denoted by $x_i$ for $i = (1,2,3,4)$, with the population expressed as a vector $X = [x_1, x_2, x_3, x_4]^T$. Then the population growth may be described by the matrix equation:

$$X(t+1) = \begin{bmatrix} x_1(t+1) \\ x_2(t+1) \\ x_3(t+1) \\ x_4(t+1) \end{bmatrix} = \begin{bmatrix} 0 & 0 & f_3 & f_4 \\ a_1 & 0 & 0 & 0 \\ 0 & a_2 & 0 & 0 \\ 0 & 0 & a_3 & a_4 \end{bmatrix} \begin{bmatrix} x_1(t) \\ x_2(t) \\ x_3(t) \\ x_4(t) \end{bmatrix} = AX(t)$$

where the $a_i$ represent the rate of individuals surviving to the next stage ($0 < a_i < 1, i = 1,2,3$ and $0 \le a_4 < 1$), and $f_3, f_4$ denote the reproductive rate of young and mature reproductives (3rd and 4th life stages) respectively. When the dominant eigenvalue ($\lambda$) of the transition matrix (A) is greater than one, then the population will grow (Cushing 1998, Caswell 2001). It is possible to relate the dominant eigenvalue to the net reproductive rate of the population, $R_0$ – that is, the number of offspring produced by one individual during the course of its lifetime. Using this approach, we incorporated life table data into the model for four economically important parasitoids: *Diachasmimorpha longicaudata* (Ashmead), *Psyttalia fletcheri* (Silvestri), *Fopius arisanus* (Sonan), and *Diaeretiella rapae* (McIntosh) [Hymenoptera:Braconidae]. For each of these parasitoid species, we simulated pesticide disturbance corresponding to incremental reductions in fecundity and survivorship. Population outcomes were then compared, revealing that the "one-size-fits-all" approach may be misleading when assessing the effects of chemical pesticides on biological control. For instance, we found that *D. rapae* was far more robust to pesticide disturbance than the other parasitoids modeled, withstanding fecundity reductions of more than 60 percent before the population declined to zero (Fig. 1). Following this approach, we also conducted sensitivity analyses to determine the relationship between different life history parameters and population outcomes of parasitoids subjected to pesticide disturbance. We discuss these results in the larger context of providing protection for economically important biological control agents exposed to pesticide sprays in the field.

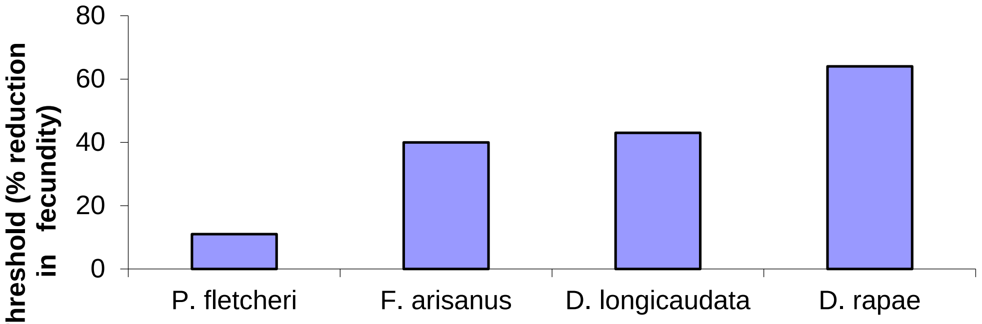

Figure 1. Extinction threshold levels of fecundity reduction for four parasitoid species, Differences between threshold levels of each of the four species highlight the potential for misleading risk assessment of one parasitoid species based on population outcomes of others. See Banks et al. (2011) for more details.

## References

Banks, J. E., L. K. Dick, H. T. Banks, and J. D. Stark. 2008. Time-varying vital rates in ecotoxicology: selective pesticides and aphid population dynamics. Ecol. Model. 210: 155–160.

Banks, J. E. and J. D. Stark. 1998. What is ecotoxicology? An ad-hoc grab bag or an interdisciplinary science? Integr. Biol. 5: 1–9.

Banks, J. E., J. D. Stark, R. I. Vargas, and A. Ackleh. 2011. Parasitoids and ecological risk assessment: Can toxicity data developed for one species be used to protect an entire guild? Biol. Control 59: 336–339.

Calow, P., R. Sibly, and V. Forbes. 2001. Risk assessment on the basis of simplified life history scenarios. Environ. Toxicol. Chem. 16: 1983–1989.

Carmo, E. L., A. F. Bueno, and R. C. O. F. Bueno. 2010. Pesticide selectivity for the insect egg parasitoid *Telenomus remus*. BioControl 55**:** 455–464.

Casas, J. 2000. *Host location and selection in the field*, pp 17–26 *In*: M.E. Hochberg and A.R. Ives (eds.): *Parasitoid Population Biology*, Princeton University Press, Princeton, NJ, USA.

Caswell, H. 2001. *Matrix Population Models: Construction, Analysis, and Interpretation. 2nd edn.* Sinauer Associates Press, Sunderland, MA, USA.

Cushing, J. M. 1998. *An Introduction to Structured Population Dynamics, Conference Series in Applied Mathematics Vol. 71*, SIAM, Philadephia, PA, USA.

Forbes, V. E., P. Calow, and R. M. Sibly. 2008. The extrapolation problem and how population modeling can help. Environ. Toxicol. Chem. 27: 1987–1994.

Godfray, H. C. J.1994. *Parasitoids, Behavioural and Evolutionary Ecology*. Princeton University Press, Princeton NJ, USA.

Jepsen, S. J., J. A. Rosenheim, and M. E. Bench. 2007. The effect of sulfur on biological control of the grape leafhopper, *Erythroneura elegantula*, by the egg parasitoid *Anagrus erythroneurae*. BioControl 52**:** 721–732.

Leslie, P. H. 1945. On the use of matrices in certain population dynamics. Biometrika 33: 184–212.

Lopes, C., A. R. R. Péry, A. Chaumot, and S. Charles. 2005. Ecotoxicology and population dynamics: Using DEBtox models in a Leslie modeling approach. Ecol. Model. 188: 30–40.

Murdoch, W. W. 1994. Population regulation in theory and practice. Ecology 75: 271–287.

Nowak, J. T., K. W. McCravy, C. J. Fetting, and C. W. Berisford. 2001. Susceptibility of adult hymenopteran parasitoids of the Nantucket pine tip moth (Lepidoptera: Tortricidae) to broad-spectrum biorational insecticides in a laboratory study. J. Econ. Entomol. 94: 1122–1127.

Stark, J. D., and J. E. Banks. 2003. Population-level effects of pesticides and other toxicants on arthropods. Ann. Rev. Entomol. 48: 505–519.

Stark, J. D., Banks, J. E, Vargas, R. 2004. How risky is risk assessment? The role that life history strategies play in susceptibility of species to pesticides and other toxicants. P. Natl. Acad. Sci. USA 101: 732–736.

Stark, J. D., R. Vargas, and J. E. Banks. 2007. Incorporating ecologically relevant measures of pesticide effect for estimating the comptability of pesticides and biocontrol agents. J. Econ. Entomol. 4: 1027–1032.

Theiling, K. M. and B. A. Croft. 1998. Pesticide side effects on arthropod natural enemies: a database summary. Agric. Ecosys. Environ. 21:191–218.

Wennergren, U., and J. D. Stark. 2000. Modeling long-term effects of pesticides on populations: beyond just counting dead animals. Ecol. Appl. 10: 295–302.

# The complicated relationship between neonicotinoid insecticides and biological control: Does it ever work or is it always bound for a disaster?

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Neonicotinoid insecticides are one of the most frequently used and the fastest growing class of pesticides in the world (Jeschke and Nauen 2008, Bass et al. 2011). These highly specific insecticides are registered for use in 120 countries (Jeschke and Nauen 2008) and annual global sales of neonicotinoids are over $1.5 billion (Ford et al. 2010), representing 17% of the total insecticide market (Jeschke and Nauen 2008). In 2010 alone, over 260,000 kg of neonicotinoid insecticides were applied to field crops, vegetables and ornamental plants in the USA (USDA 2012). The ubiquity of these systemic insecticides stems from their excellent efficacy (Elbert et al. 2008), long activity in plant tissues (Raupp et al. 2004), and a wide variety of formulations that allow for spraying directly on plants, drenching into the soil through irrigation systems, injections into tree trunks, and applications to seeds of agricultural crops before they are planted (Elbert et al. 2008).

Neonicotinoid applications, however, may have negative environmental effects. In particular, these insecticides have been associated with severe outbreaks of many species of an unsusceptible herbivore, spider mites (Trombidiformes: Tetranychidae) on a wide variety of trees, shrubs and crop plants (Sclar et al. 1998, Raupp et al. 2004, Gupta and Krischik 2007, Szczepaniec et al. 2011). One of the mechanisms that have been proposed to contribute to these outbreaks is elimination of natural predators of spider mites such as predatory mites (Mesostigmata: Phytoseiidae), minute pirate bugs (Hemiptera: Anthocoridae), and spider mite destroyers (Coleopetera: Coccinellidae). In separate studies conducted in urban and field crop systems, we examined how neonicotinoid insecticides applied in formulations and doses specific to these systems affected communities of predatory mites and insects associated with elm trees, boxwood shrubs, and cotton plants. We also documented the effect of the insecticides on populations of spider mites. While spider mites were more abundant on plants exposed to these insecticides in all of the plant systems, we found that the effect of neonicotinoid insecticides on abundance of predators of spider mites varied among plants. In the three-year study that measured the effect of imidacloprid on predators of spider mites associated with elm trees, the most abundant predators, *Galendromus spp.* (Mesostigmata: Phytoseiidae) were less abundant on imidacloprid-treated trees in some but not all years. On boxwoods and cotton, on the other hand, predators of spider mites were present in low numbers independently of the treatments, and were not affected by applications of the neonicotinoid insecticides. We also found that the general composition of predators of spider mites associated with each plant type varied depending on the system, and the relative importance of predators in suppressing spider mites in each of these systems independently of insecticide applications is likely highly variable.

While we didn't find unequivocal evidence for neonicotinoid-driven elimination of predators as a mechanism driving outbreaks of spider mites, dampened top-down suppression of spider mites may have contributed to outbreaks of these herbivores in at least one plant system. Consequences of widespread and continuous applications of insecticides on predators are frequently difficult to establish, and long-term field experiments are essential to accurately measure the impact of neonicotinoid insecticides on predators and the ecosystem services they provide.

## References

Bass, C., A. Puinean, M. Andrews, P. Cutler, M. Daniels, J. Elias, V. Paul, A. Crossthwaite, I. Denholm, L. Field, S. Foster, R. Lind, M. Williamson, and R. Slater. 2011. Mutation of a nicotinic acetylcholine receptor beta subunit is associated with resistance to neonicotinoid insecticides in the aphid Myzus persicae. BMC Neuroscience 12: 51.

Elbert, A., M. Haas, B. Springer, W. Thielert, and R. Nauen. 2008. Applied aspects of neonicotinoid uses in crop protection. Pest Manag. Sci. 64: 1099–1105.

Ford, K. A., J. E. Casida, D. Chandran, A. G. Gulevich, R. A. Okrent, K. A. Durkin, R. Sarpong, E. M. Bunnelle, and M. C. Wildermuth. 2010. Neonicotinoid insecticides induce salicylate-associated plant defense responses. P. Natl. Acad. Sci USA 107: 17527–17532.

Gupta, G., and V. A. Krischik. 2007. Professional and consumer insecticides for management of adult Japanese beetle on hybrid tea rose. J. Econ. Entomol. 100: 830–837.

Jeschke, P., and R. Nauen. 2008. Neonicotinoids - from zero to hero in insecticide chemistry. Pest Manag. Sci. 64: 1084–1098.

Raupp, M. J., R. E. Webb, A. Szczepaniec, D. Booth, and R. Ahern. 2004. Incidence, abundance, and severity of mites on hemlocks following applications of imidacloprid. Journal of Arboriculture 30: 108–113.

Sclar, D. C., D. Gerace, and W. S. Cranshaw. 1998. Observations of population increases and injury by spider mites (Acari : Tetranychidae) on ornamental plants treated with imidacloprid. J. Econ. Entomol. 91: 250–255.

Smith, J. F. 2010. Early-season management of twospotted spider mite on cotton and impacts of infestation timing on cotton yield loss. Ph.D., Mississippi State University Starkville, MI.

Szczepaniec, A., S. F. Creary, K. L. Laskowski, J. P. Nyrop, and M. J. Raupp. 2011. Neonicotinoid Insecticide Imidacloprid Causes Outbreaks of Spider Mites on Elm Trees in Urban Landscapes. PLoS ONE 6(5): e20018.

USDA, N. A. S. S. Agricultural Chemical Use Database. http://www.pestmanagement.info/nass/ Accessed May 2012.

# RNAi-based insecticidal crops: Potential effects on non-target species

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RNAi is a sequence specific mechanism that silences protein production when particular mRNAs are bound and enzymatically cleaved. Genetically modified crops that silence critical gene function in insect pests have been developed, and are a likely future direction for commercial pest management. Potential hazards posed by RNAi-based crops include 1) off-target gene silencing in non-target organisms, 2) silencing the target gene in non-target organisms 3) immune stimulation, and 4) saturation of the RNAi machinery. Although steps can be taken to reduce the likelihood of accidental binding of small RNAs to unintended gene targets, evidence suggests that off-target and non-target binding and gene suppression may be widespread in RNAi. Furthermore, the large scale adoption of currently available GM crops suggests that the number of species that will be exposed to RNAi-based GM crops will be extensive, although their degree of exposure and risk requires additional research. Specific areas that warrant future work include the following: 1) The persistence of small RNAs associated with GM plants in the environment is largely unknown; 2) there is poor resolution of crop-based food webs and additional information regarding which species will be exposed to RNAi-based crops is needed; 3) genomic information for most species is absent and limits our ability to understand which species may be affected by RNAi; and 4) it is unclear whether laboratory testing that only focuses on toxicity can predict the effects of sublethal gene silencing produced by RNAi. Ultimately, the costs and benefits of pesticidal RNA must be considered relative to current pest management options.

# Session 16: Pest Management for an Urbanizing World: Arthropod Biological Control in Ornamental Landscapes

## The value of vacant land: arthropod predators and biocontrol services in changing city landscapes

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The majority of our global population lives in cities. As our population continues to grow, we will not regain large undisturbed habitats to preserve declining species, restore ecosystem functions, or support ecosystem services. Therefore, human-mediated urban habitats need to serve multiple functions, from conservation to food production. Due to economic decline and the recent foreclosure crisis, vacant land has become a significant landscape element in several U.S. cities. A recent resurgence of interest in local food production has the potential to dramatically reshape the composition of urban green space. Sustainable food production within urban landscapes requires arthropod populations able to support adequate arthropod-mediated ecosystems services including biocontrol services supplied by generalist predators. This study examined how the conversion of urban vacant land to cropland influenced the composition of the generalist predator community and the level of biological control services found within habitat patches. Our hypothesis was that the increased habitat disturbance generated through the establishment and maintenance of a community garden would result in reduced populations of arthropod generalist predators and lesser biological control services. We found that the abundance or activity density measured for the majority of arthropod predators, as well as levels of predatory activity were either conserved or enhanced within community gardens. However, the predators Dolichopodidae, Linyphiidae, and Opiliones declined within garden patches which may have implications for pest management.

### References

Gardiner, M. M., C. E. Burkman, and S. P. Prajzner. The value of vacant land for arthropods and the ecosystem services they support in changing urban landscapes. Environ. Entomol., Invited Forum Article (submitted).

Gardiner, M.M., S.P. Prajzner, C.E. Burkman, S. Albro, and P.S. Grewal. Vacant land conversion to community gardens: influences on generalist arthropod predators and biocontrol services in the urban landscape. Urban Ecosystems (submitted).

# Hot in the city: effects of urban heat on the ecology and evolution of urban forest pests

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Many herbivorous arthropods, particularly tree pests, are more abundant and damaging in urban than rural areas. Urbanization has many effects on ecosystems but the urban heat island effect is one of the most pervasive. However, no research has examined how urban heat affects the fitness and abundance of herbivorous arthropods. The overall hypothesis of this research is that warm habitats increase herbivore abundance due to direct physiological benefits and a breakdown of trophic dynamics wherein sedentary herbivores acclimate or adapt to high urban temperatures but their mobile predators and parasitoids do not, resulting in high herbivore fitness and less effective natural enemy communities. We test this hypothesis with survey and manipulative experiments that focus on two common scale insect pests, *Parthenolecanium quercifex* (Fitch) (Hemiptera: Coccidae) and *Melanaspis tenebricosa* (Comstock) (Hemiptera: Diaspidae) and their natural enemies. Our objectives were to determine if temperature affects: 1) herbivore abundance and community structure, and 2) natural enemy abundance, community structure and efficacy in hot and cool parts of cities, across a latitudinal gradient. These relatively unstudied mechanisms by which heat could trigger pest outbreaks have important new implications for predicting the health of urban and natural forests under warmer future conditions.

To determine how temperature affects herbivore and natural enemy communities we sampled scale insects and other herbivores on red maple, *Acer rubrum* L. (Sapindaceae), and willow oak, *Quercus phellos* L. (Fagaceae), trees in the hottest and coolest parts Raleigh, North Carolina, USA. We also sampled red maples in the hottest and coolest parts of Baltimore, Maryland, New York, New York, and Boston, Massachusetts USA. We overlaid a surface temperature map of each city with a map of street trees in Arc GIS. We visited 20 willow oak and 30 red maple trees in the hottest and coolest pixels of the map (hereafter hot and cold sites respectively) and counted *P. quercifex* and *M. tenebricosa* on 4 branches per tree. We hung sticky cards in each tree for 5 days to passively sample the natural enemy community. We also collected branches from each tree in Raleigh to rear parasitoids from each scale species.

Our research indicates that, in the hottest parts of cities, the abundance of some scale species is greater than in cooler parts. Mechanisms for this may include changes in natural enemy community structure and efficacy or phenotypic changes in scale insects in response to higher temperatures. Detailed results will be presented in other publications. These effects may serve as a canary in the coal mine and predict greater pest outbreaks in response to global warming. To protect urban and natural forests, scientists need a clear understanding of how rising temperatures affect herbivore ecology and evolution.

# Chemical and nutritional ecology of *Ibalia leucospoides ensiger* (Norton) (Hymenoptera: Ibalidae), a parasitoid of siricid wasps

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*Sirex noctilio* Fabricius (Hymenoptera: Siricidae) is a recently-established, exotic pest of pines in the Great Lakes region in North America (Hoebeke et al. 2005, Dodds et al. 2010). Soon *S. noctilio* may invade southern forests in the USA, and attack pines of major economic importance, particularly species for which *S. noctilio* has proven to be a serious pest in other continents. We are developing trapping technologies for hymenopteran parasitoids, especially *Ibalia leucospoides ensiger* (Norton) (Hymenoptera: Ibalidae) that are known to be effective biological control agents of the exotic woodwasp in other non-native habitats (Murphy 1998). Our research objectives are as follows: 1) to assess olfactory attractants for parasitoids of *S. noctilio* that could be effectively used as baits in traps, 2) to determine the efficacy of different trapping designs for parasitoid species of *S. noctilio*, and 3) to assess effects of sugar feeding on parasitoids in the laboratory. We are currently conducting experiments on assessing the responses of *I. leucospoides ensiger* to various olfactory cues such as fungal cultures, venom gland, tree volatiles, and odors from adult *Sirex*. In the Fall of 2011, we conducted feeding bioassays on adult *I. leucospoides ensiger* where we assessed factors affecting their survival and reproduction. We determined the longevity, survivorship, and reproductive success of *I. leucospoides ensiger* if provided with different types of sugars such as glucose, sucrose, fructose, maltose, and lactose (distilled water was used as a control). Two levels of molarity were used: 0.5 and 2 M for each sugar-type. Preliminary results indicate that greatest longevity was found in *I. leucospoides ensiger* females when they were provided with fructose, glucose, and sucrose with the lowest longevity with maltose solution. There were no differences in number of eggs produced by females when provided the five sugar-types, although there was a trend of greater number of eggs produced by adults with sucrose treatment. Overall, our study may assist in providing information about how, where, and when to collect these biocontrol agents in the wild, in addition to maintaining viable laboratory colonies for control efforts of *S. noctilio*.

## References

Dodds K. J., P. de Groot, and D. A. Orwig. 2010. The impact of *Sirex noctilio* in *Pinus resinosa* and *Pinus sylvestris* stands in New York and Ontario. Can. J. Forest Res. 40: 212–223.

Hoebeke E. R., D. A. Haugen, and R. A. Haack. 2005. *Sirex noctilio*: Discovery of a Palearctic siricid woodwasp in New York. News. Michigan Entomol. Soc. 50: 24–25.

Murphy, S. T. 1998. Indigenous siricid spp. parasitoid communities and principal biological control agents of *Sirex noctilio* in Australasia. pp. 31-35 *In* E. Tadeu Iede, E. Schaitza, S. Penteado, R. C. Reardon, and S. T. Murphy (eds.) Proceedings of a conference: training in the control of *Sirex noctilio* by the use of natural enemies. Colombo: EMBRAPA-CNPF, United States Department of Agriculture, Forest Service, Forest Health Tecnhnology Enterprise Team, Morgantown, West Virginia.

# Biological control in native vs. exotic urban ornamental landscapes: colonization of experimental backyards by parasitic Hymenoptera

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There is heightened interest in effects that the provenance of plants in the landscape has on animals inhabiting them. This question is of great interest for urban ornamental landscapes, which tend to be mosaics of native and exotic plants. There is a considerable body of research on insect herbivores (e.g. Farrell *et al.* 1992, Keene and Crawley 2002, Burghardt et al. 2010), but little attention has been paid to arthropod natural enemies. In eastern North America, many commonly grown exotic woody plants were missing from the continent for millions of years prior to the arrival of Europeans. We present the hypothesis that due to the lack of a recent co-evolutionary history with these plants, native natural enemies will be less well able to utilize the resources – architectural features and nutritional supplements – provided by exotic plants than they will those of native plants, and hence be less numerous and diverse in landscapes dominated by exotic plants. We test this in a replicated experiment comprising 0.08 Ha plots planted to congeners of 15 genera of woody plants from either Eurasia or North America, grown in a turf of tall fescue. Adult hymenopterous parasitoids collected by vacuum sampling from the turf were statistically less abundant, and exhibited statistically lower species richness, in the exotic plots, supporting the hypothesis. The parasitoid complex comprised 43 taxa of broad taxonomic and host affinities. At least some of these animals parasitize herbivores that feed on woody plants in the landscape, and probably visit the turf to take advantage of plant-provided nutritional supplements.

## References

Burghardt, K. T., D. W. Tallamy, C. Phillips, and K. J. Shropshire. 2010. Non-native plants reduce abundance, richness, and host specialization in lepidopteran communities. Ecosphere 1: 1–22.

Farrell, B. D., M. Mitter, and D. J. Futuyma.1992. Diversification at the insect-plant interface. BioScience 42: 34–42.

Keene R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. T. Ecol. Evol. 17: 164–170.

# Arthropod communities in native vs. alien urban landscapes: how do they differ?

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Urbanization is occurring at a rapid rate worldwide. With increasing urbanization come alterations in land use patterns and concerns of decline in biodiversity and reduced ecosystem function. Of particular interest is how common features of urban landscapes affect biodiversity of arthropod communities, in terms of richness, abundance, and ecosystem services, and result in outbreaks of herbivorous arthropods (Raupp et al. 2010). A better understanding of the effects of urbanization on community dynamics is critical towards sustaining and restoring ecosystem function in urban environments. A common feature of urbanization is an increase in the use of alien (non-native) plants relative to native plants as you move along the urbanization gradient, i.e. more urban, more alien plants (McDonnell and Pickett 1990). Of the little work that has addressed the influence of plant origin on arthropod communities, most has focused on herbivorous arthropods and fewer on arthropod natural enemies. We investigate the hypothesis that urban landscapes of native plants will sustain a greater diversity of insect herbivores, and thus their natural enemies, than landscapes of alien plants that lack an evolutionary history with native arthropods. This study used simulated urban landscapes to compare the density and diversity of phytophagous insects and their natural enemies in paired plots comprised entirely of native or alien woody ornamental plants. Arthropods were sampled over 3 years and identified to species (or morpho-species), and assigned to their appropriate trophic or feeding guild. Comparisons were made of species richness and abundance between native and alien landscapes. Results of this study found that the response of arthropod communities were guild specific. There was increased colonization of species over time (3 years) for all guilds, but colonization was greater in the native than alien landscapes for chewing herbivores and parasitoids. The abundance of chewing herbivores was greater in native landscapes than alien, whereas sucking herbivores did not differ in abundance between native and alien landscapes. Predators responded similarly to native and alien landscapes, whereas parasitoids responded positively to native landscapes. This study provides insight into herbivore and natural enemy responses to plant origin and information towards the development of measures to conserve natural enemies and the ecosystem service of biological control they provide in urban environments.

## References

McDonnell, M. J., and S. T. A. Pickett. 1990. Ecosystem structure and function along urban-rural gradients: an unexploited opportunity for ecology. Ecology 71: 1232–1237.

Raupp, M. J., P. M. Shrewsbury, and D. A. Herms. 2010. Ecology of herbivorous arthropods in urban landscapes. Ann. Rev. Entomol. 55: 19–38.

# Session 17: Evaluation and Practical Application of Parasitoids and Predators of the Tomato Leaf Miner *Tuta absoluta*

## ***Nesidiocoris tenuis*** (Reuter) (Hemiptera: Miridae) as the basis for control ***Tuta absoluta*** (Meyrick) (Lepidoptera: Gelechiidae) in tomato protected crops in Spain

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The tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is one of the most devastating pests of tomato crops in South America (Miranda et al. 1998). Since its introduction in Europe at the end of 2006 it has spread quickly throughout the Mediterranean Basin (Desneux et al. 2010), causing very serious damage to tomato crops in different countries. In its original area of distribution, this pest has several natural enemies (Desneux et al. 2010). However, the pest arrived in Europe without them.

*Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) is a polyphagous mirid that commonly appears in tomato and other agricultural crops and natural vegetation in the Mediterranean region and the Canary Islands (Goula and Alomar 1994; Carnero et al. 2000; Trottin-Caudal et al. 2006). *N. tenuis* is known as an effective natural enemy of whitefly (Sánchez and Lacasa 2008; Calvo et al. 2009) and was demonstrated as a good biological control agent against *T. absoluta* eating their eggs and small larvae (Urbaneja et al. 2009).

### Improved pre-planting release strategy with *N. tenuis*

For an efficient control of the pest, the predatory bug must be present before the pest appearance and in sufficient numbers on the plants since the first weeks after planting (Urbaneja et al. 2009). Koppert developed a predator-in-first strategy for *N. tenuis* in tomato (Calvo et al. 2010, 2012a) which allow establishing the predator just after transplanting. This strategy consists in the release of the predator adults in the nursery trays, several days before planting, and adding *Ephestia kuehniella* eggs as food source before prey, *T. absoluta* or whitefly, availability. The aim of this novel approach is to ensure the presence of *N. tenuis* nymphs on the plants in the crop, as soon as possible, and as uniformly spread as possible, to prevent the hatch of *T. absoluta* eggs and consequent infestations of leaves, and for the control of whiteflies. This strategy has been tested in semi-field and field trial and has been demonstrated very effective against *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae) and *T. absoluta.*

In addition to the implementation of the use of this strategy with the predatory bug against *T. absoluta*, several other studies has been conducted to look for and assess natural enemies since the invasive pest was detected in Europe. Thus, some parasitoids from the Mediterranean basin have been found attacking *T. absoluta* (Cabello et al. 2009; Desneux et al. 2010; Zappalá et al. 2012). Among these parasites, the egg parasitoid *Trichogramma achaea* Nagaraja and Nagarkatti (Hymenoptera: Trichogrammatidae) and the eulophid *Necremnus artynes* (Walker) (Hymenoptera: Eulophidae), have been studied and evaluated (Cabello et al. 2009; Calvo et al. 2012b, 2012c) as candidates for the control of *T. absoluta* in tomato crops

### Simplifying the biological control strategy against *T.absoluta*

Complementary trials that we have carried out to assess the efficacy of some parasitoids of *T.absoluta* in semi-field trials, showing a reduction in the damages caused by the pest larvae in leaves and frutis. For example, in semi-field trials, the release of *N. artynes* at 1 and 2 ind./m$^2$ (x7 weeks) significantly reduced the percentage of attacked fruits by *T. absoluta*, specially with the higher rate of 2 ind./m$^2$. However the percentage of reduction achieved is not still tolerable in practice. This fact, together with the estimated production cost for *N. artynes* would suggest that *N. artynes* should be combined with other control agents in order to reduce its total release rate (Calvo et al. 2012c).

When these biological control agents were used simultaneously with *N. tenuis*, their contribution did not improve significantly the efficacy of *N. tenuis* used alone in the pre-planting release strategy. In a trial carried out in 2011 using *T. achaea*, *Bacillus thuringiensis* and *N. tenuis*, alone or mixed with the others biological control agents, the mean percentage of leaf area affected by *T. absoluta* increased in the control treatment reaching an average of 95,2 ± 0,8% at the end of the study. In the rest of treatments (*N. tenuis* + *T. achaea*, *N. tenuis*, *N. tenuis* + Bt) the mean percentage was maintained below 17% of affected leaf area by *T. absoluta* during the whole experiment, but without significant differences between treatments of the different strategies. Similar results were obtained when *N. artynes* was used with *N. tenuis*. All treatments including *N. tenuis* significantly reduced the percentage of mined area by *T. absoluta* in respect to the treatment with the pest only, where more than 90% of the leaf surface was consumed by the pest at the end of the experiment. The combination of 1 *N. artynes*/m$^2$ per week for 5 weeks and the standard application of *N. tenuis* resulted as effective as the pre-plant application of *N. tenuis* alone. The lowest percentage of mined area by *T. absoluta* was observed in plots combining the pre-plant application of *N. tenuis* with additional releases of 1 *N. artynes*/m$^2$ per week for 5 weeks. In all treatments receiving *N. tenuis* the percentage of fruits attacked by *T. absoluta* were significantly lower in respect to the treatment with the pest only.

Although these parasitoids and probably other biological control agents could help in the control of the tomato borer in those circumstances in which *N.tenuis* could not reach enough numbers to sufficiently control the pest, the biological control strategy of this pest must be conducted by the simplest and cheaper strategy. The current implementation and rate of success of the pre-planting release method of *N.tenuis* in commercial greenhouses grown tomato after two years from its first use in practice, has conducted to an extension of the surface of protected tomato crops in Spain using biological control of pest. Actually this strategy is the most commonly used in tomato crops in Spain and also in other Mediterranean countries (Calvo et al. 2012c).

## References

Cabello,T., J.R. Gallego, E. Vila, A. Soler, M. del Pino, A. Carnero, E. Hernández-Suárez, and A. Polaszek. 2009. Biological control of the South American tomato pinworm *Tuta absoluta* (Lep.: Gelechiidae), with releases of *Trichogramma achaeae* (Hym.: Trichogrammatidae) in tomato greenhouses of Spain. IOBC/WPRS Bull. 49: 225–230

Calvo, F.J., K. Bolckmans, P. Stansly, and A. Urbaneja. 2009. Predation by *Nesidiocoris tenuis* on *Bemisia tabaci* and injury to tomato. BioControl 54: 237–246.

Calvo, F.J., A. Giménez, and J.E. Belda. 2010. Una nueva estrategia para el control biológico de mosca blanca y *Tuta absoluta* en tomate. Phytoma España, 216: 46–52.

Calvo, F.J., K. Bolckmans, and J.E. Belda. 2012a. Release rate for a pre-plant application of *Nesidiocoris tenuis* for *Bemisia tabaci* control in tomato. BioControl. In press.

Calvo, F.J., M.J. Lorente, P.A. Stansly, and J.E. Belda. 2012b. Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisa tabaci* in greenhouse tomato. Entomol. Exp. Applic. 143:111–119.

Calvo, F.J., J. Soriano, K, Bolckmans, and J.E. Belda. 2012c. A successful method for whitefly and *Tuta absoluta* (Meyrick) control in tomato. Evaluation after two years of application in practice. IOBC/WPRS Bull. 80: 237–244.

Carnero, A., S. Díaz, S. Amador, M. Hernández, and E. Hernández. 2000. Impact of *Nesidiocoris tenuis* (Heteroptera, Miridae) on whitefly populations in protected tomato crops. IOBC/WPRS Bull. 23: 259.

Desneux, N., E. Wajnberg, K. A. G. Wyckhuys, G. Burgio, S. Arpaia, C. A. Narváez-Vasquez, J. González-Cabrera, D. Catalán Ruescas, E. Tabone, J. Frandon, J. Pizzol, C. Poncet, T. Cabello, and A. Urbaneja. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. J. Pest. Sci. 83:197–215.

Goula, M., and O. Alomar. 1994. Míridos (Heteroptera: Miridae) de interés en el control integrado de plagas en tomate. Guía para su identificación. Bol. Sanid. Veg. Plagas 20:131–143.

Miranda, M. M. M., M. Picanco, J. C. Zanuncio, and R. N. C. Guedes. 1998. Ecological life table of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Biocon. Sci. Technol. 8: 597–606.

Sánchez J. A., and A. Lacasa. 2008. Impact of the zoophytophagous plant bug *Nesidiocoris tenuis* (Heteroptera: Miridae) on tomato yield. J. Econ. Entomol. 101:1864–1870.

Trottin-Caudal, Y., C. Fournier, J. M. Leyre, and C. Chabriere. 2006. La tomate sous serre dans le Sud-Est de la France. Protection contre la punaise *Nesidiocoris tenuis*. Infos Ctifl 224: 30–35.

Urbaneja, A., H. Montón, and O. Mollá. 2009. Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus caliginosus* and *Nesidiocoris tenuis*. J. Appl. Entomol. 133: 292-296.

Zappalá, L., U. Bernard, A. Biondi, S. Deliperi, G. Delrio, M. Giorgini, P. Pedata, C. Rapisarda, G. Tropea Garzia, and G. Siscaro. 2012. Recruitment of native parasitoids by the exotic pest *Tuta absoluta* (Meyrick) in Southern Italy. Bull. Insectol. 65: 61–65.

# Biological control of *Tuta absoluta*: sampling and evaluation of new hemipteran predators found in Brazil

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Tomato (*Lycopersicon esculentum* Mill.), originating in South America, is now grown almost everywhere in the world and its global production had doubled in the last 20 years. Brazil occupies a prominent place in world production with a tomato area of 57,600 ha and production of about three million tons (Agrianual 2010).

Insect pests significantly reduce the yield of tomato crop in Brazil and worldwide. Among the major pests that attack tomatoes, is the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). This pest, in high and uncontrolled infestations, can destroy up to 90% of leaf area, and damage the stems and fruit (Souza and Reis 1992). In Brazil, two other important lepidopteran pests occur in tomato: the tomato fruit borer, *Neoleucinodes elegantalis* (Lepidoptera: Crambidae) and the tomato fruitworm *Helicoverpa zea* (Lepidoptera: Noctuidae). Thrips, whiteflies, aphids and mites are also problematic (Bueno et al. 2012).

Chemical control by applications of pesticides is the main practice used to minimize pest damage. In some cases, up to 36 sprays are done per crop cycle of 12 weeks (Guedes and Picanço 2011). Thus, development of resistance to these pesticides is usually fast (Siqueira et al. 2000), resulting in ineffectiveness and a strong reduction of natural enemy species of the various pest organism, as well as in high pesticide residue levels on the harvested tomato fruits.

Therefore, search for alternatives of chemical pesticides is of high priority. We think that biological control is a desirable solution to control *T. absoluta* and other pests on tomato crops, and that it is best to concentrate to search on predators, because they might be able to control *T. absoluta* as well as several of the other pests occurring on Brazilian tomato crops.

Mirid predatory bugs are currently used with success in Europe to control *T. absoluta* and other pests (Calvo et al. 2012). Hemipteren predators are currently popular organisms for biological control compared with 20 years ago (Bueno and van Lenteren 2012; van Lenteren 2012).

A survey of the predatory bugs from the family Miridae was carried out in tomato and tobacco crops located in the municipalities of Ribeirao Vermelho and Lavras, Minas Gerais State, Brazil. The number of nymphs and adults ranged from 0 to 8 per plant per sampling date, and 95% of the plants had at least one nymph and/or adult predator. Three predatory bug species (Miridae: Diciphini) were found on tobacco: *Campyloneuropsis infumatus*, *Engytatus varians* and *Macrolophus* basicornis (Bueno et al. 2013).

After identification, the field collected nymphs and adults were kept on tobacco plants (*Nicotiana tabacum* L.) in ventilated acrylic cages in a climate room at 25 ± 2ºC, RH 70 ± 10% and 12 photophase, and fed with eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae). Preliminary experiments on search capacity, predation, survival and reproduction on tomato were done with the three mirid predators and also with the heteropterans predatory bugs *Geocoris punctipes* and *Orius insidiosus*.

Direct observations have shown that the three predatory mirid species and the geocorid *G. punctipes* are able to walk and search without difficulties on leaflets and stems of tomato plants (Figure 1). This is an important finding, as nymphs and adults of *O. insidiosus* could not walk on the stem of the tomato plant (Figure 2), although the female laid eggs in the longitudinal veins and in the petiole of the tomato leaflets. All three predatory mirids can easily reproduce on tomato plants.

Figure 1. Mirid predatory bug on tomato plants (Laboratory of Biological Control, UFLA, Brazil)

Figure 2. *Orius insidiosus* on the stem of tomato plant (Laboratory of Biological Control, UFLA, Brazil)

All five predators find *T. absoluta* eggs and larvae and use them as prey. A high egg predation capacity of more than 90 eggs per 24 h of *T. absoluta* took place by *E. varians* and *M. basicornis; C. infumatus* and *O. insidiosus* have egg predation rates of on average 50 and 30 eggs per day, respectively. The predatory bug *G. punctipes* is a very poor egg predator with a consumption of only 0.5 eggs per 24 h on average. Arno et al. (2009) found egg predation rates of over 100 eggs by adults of *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae), both mirid predators used in the Mediteranean basin as biological control agents of *T. absoluta* and witheflies in greenhouse tomato crops, the former since 1994 and the latter since 2003, and both in large scale in the market (van Lenteren 2012).

Interestingly, the mirids *G. punctipes* and *O. insidiosus* are all capable of preying on larvae of *T. absoluta* by stinging through the leaf epidermis into the prey within the mine. *G. punctipes* preyed at least 9 *T. absoluta* larvae per day, and had preference for the 1st and 2nd instars.

The developmental time from egg-adult of *C. infumatus*, *E. varians*, *M. basicornis , O. insidiosu*s and *G. punctipes*, respectively, were 34, 34, 38, 25 and 69 days at 20 ±1°C. The fecundity of *C. infumatus*, *E. varians*, *M. basicornis*, *O. insidiosus* and *G. punctipes* were, respectively, 106, 92, 97, 169 and 42 eggs/female at the same temperature. The developmental time of the three mirid predatory bugs in tomato plants is 22 to 25 days at 25 ±2°C.

Based on our findings, we conclude that *O. insidiosus* is not a suitable candidate for control of *T. absoluta*, because it is not able to walk on tomato, sticks to the glandular hairs and dies. *Geocoris punctipes* seems also not suitable, because it shows poor egg predation, high immature mortality and a long developmental time. The three mirid predators seem to be the best candidates for control of *T. absoluta* with their high egg predation and good survival on tomato.

We currently have a large scale research program running to determine the biological control capabilities of the three new mirid predators of *T. absoluta*. Next to their developmental time, reproduction capacity, predation capacity of all nymphal stages and the adult stage, we also measure the quality of these predators for controlling two other lepidopteran pests on tomato and for control of whiteflies, aphids, and leaf miners. Further, prey preference studies are planned and a mass rearing for each of the three species is in development.

## References

Agrianual. 2010. FNP. Consultoria e comércio. Anuário da Agricultura Brasileira.

Arno, J., R. Sorribas, M. Prat, M. Matas, C. Pozo, and D. Rodrigues. 2009. *Tuta absoluta* a new pest in IPM tomatoes in the Northeast of Spain. IOBC/WPRS Bull. 49: 203–208.

Bueno, V. H. P., and J. C. van Lenteren. 2012. Predatory Bugs (Heteroptera), pp. 539–569. *In* A. R. Panizzi, and J. R. P. Parra (eds.), Insect Bioecology and Nutrition for Integrated Pest Management. CRC Press,Boca Raton, FL, USA.

Bueno, V. H. P., F. C. Montes, A. M. Calixto, J. C. Lins Jr., and J. C. van Lenteren. 2012. Can recently found Brazilian hemopteran predatory bugs control *Tuta absoluta*? IOBC/WPRS Bull. 80: 63–67

Bueno, V. H. P., J. C. van Lenteren, J. C. Lins Jr., A. M. Calixto, F. C. Montes, D. B. Silva, L. D. Santiago, and L. M. Pérez. 2013. New records of *Tuta absoluta* (Meyrick) (Leidoptera: Gelechiidae) predation by Brazilian Hemipteran predatory bugs. J. Appl. Entomol. 137: 29–34.

Calvo, J. F., K. Bolckmans, and J. E. Belda. 2012. Release rate for a pre-plant application of *Nesidiocoris tenuis* for *Bemisia tabaci* control in tomato. Biocontrol DOI: 10.1007/s10526-012-9455-1.

Guedes, R.N.C., and M. Picanço. 2011. *Tuta absoluta* in South America: pest status, management and insecticide resistance. EPPO/IOBC/FAO/NEPPO Joint International Symposium on Management of *Tuta absoluta* (tomato borer). Agadir, Marocco, Nov. 16-18, 2011, 15–16.

Siqueira H. A., R. N. Guedes, and M. C. Picanço. 2000. Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). Agric. For. Entomol. 2: 147–153.

Souza, J.C. de., and P. R. Reis.1992. Traça-do-tomateiro, histórico, reconhecimento, biologia, prejuízos e controle. Belo Horizonte: EPAMIG Boletin 32, 19p.

van Lenteren, J. C. 2012. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl 57: 71–84. DOI: 10.1007/s10526-011-9395-1.

# Impact of interspecific competition with an omnivorous predator on survivorship of an ectoparasitoid of *Tuta absoluta* in tomato agro-ecosystem.

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Over the past decades, biological control of arthropod pests has become steadily a sustainable alternative to pesticides (Pimentel 1991, 2005), notably in greenhouse crops. However, Integrated Pest Management packages often rely on the simultaneous use of two or more biological control agents (e.g. parasitoids and generalist predators). Unfortunately, the possible occurrence of direct and/or indirect interactions among natural enemies may reduce the overall efficacy of biological control agents (Rosenheim et al. 1995; Chailleux et al. 2013). The less competitive biological control agents may be excluded from the agroecosystem if there are not sufficient ecological niches (Gause 1934). Therefore, identification of such interactions is of primary importance to the success of biological control programs (Bogran et al. 2002; Messelink et al. 2011). How natural enemy populations coexist while competing for the same prey is one of the key questions in ecology (Chase and Leibold 2003; Finke and Snyder 2008). We present a study assessing competitive interactions between two natural enemy species: an ectoparasitoid and an omnivorous predator that attack a major invasive pest in Europe on tomato, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) (Desneux et al. 2011).

Biological control is a key component of integrated pest management programs in tomato greenhouses in Europe (Pilkington et al. 2010). The omnivorous mirid predator, *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae), is the predator most commonly encountered in tomato crops in Europe. Its ability to suppress whitefly populations is considered to be important for biological control in protected crops (Lykouressis et al. 2009; Castañé et al. 2004). It is also efficient as a biological control agent of *T. absoluta* though most of the predation occurs on eggs, i.e. letting larval stages escape predation (Desneux et al. 2010). So far, no biological control agents specifically targeting *T. absoluta* larval stages have been available to producers (Chailleux et al. 2012). Thus, damage to the crop is impossible to prevent when *T. absoluta* larvae are abundant in the greenhouse. Parasitoids have been reported as ecto- or endoparasitoids (Eulophidae, Braconidae and Ichneumonidae) of *T. absoluta* in the Mediterranean basin (Desneux et al. 2010; Urbaneja et al. 2012; Zappala et al. 2012). Among those recorded, the idiobiont ectoparasitoid *Stenomesius japonicus* (Ashmead) (Hymenoptera: Eulophidae) preferentially parasitizes older larvae (3rd and 4th instars) the stages that inflict the most damage to the plant. It may be possible to use *S. japonicus* through inoculative releases for biological control purposes though its capacity to sustain populations in tomato crops in the presence of *M. pygmaeus* should be assessed (potential intraguild predation [IGP] or competition).

The objectives of our study were to: 1) detect potential interspecific competition between the predator and the parasitoid, 2) identify resource utilization patterns that may promote coexistence of the two natural enemies, and 3) assess the impact of tentative interactions on overall control of *T. absoluta*. The predator, *M. pygmaeus*, was expected to be a superior competitor since omnivorous predators are supposed to sustain populations despite overall low levels of prey. The parasitoid was expected to suffer resource competition and/or IGP. A long-term experiment was carried out in cages in greenhouse compartments located in Sophia-Antipolis (French Riviera, France). Each cage contained six tomato plants watered automatically with a nutritive solution. The two natural enemies were released alone or in combination in the cages. Low and high densities of *T. absoluta* were tested. Each species were released twice: an initial release and a second one at the half way mark of the species life cycle (to avoid cycling populations). *Macrolophus pygmaeus* were released first, two weeks before the prey. *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs were placed on the plants to mimic an inoculative release of the predator (as advised by biological control companies). We recorded population dynamics every week for a total of eight weeks.

*Stenomesius japonicus* and *M. pygmaeus* established in each treatment in which they were released and were present throughout the course of the study. For both biological control agents, their populations were significantly lower when in competition vs. when alone. However, the highest impact on *T. absoluta* populations was still observed when the two natural enemies were together. Contrary to our initial hypothesis, the omnivorous predator did suffer resource competition with the parasitoid. We demonstrated that coexistence of an omnivorous predator and a parasitoid is possible despite occurrence of a two-way negative indirect interaction.

In addition, laboratory observations showed that the predator behaved as a kleptoparasite on *T. absoluta* larvae previously paralyzed by the parasitoid. Most of time, this led to the death of parasitoid juveniles despite the lack of actual direct predation on the parasitoid larvae. Such kleptoparasitism, being very similar to IGP (at least in its effect on parasitoid larvae) undoubtedly increases the risk of parasitoid exclusion, i.e. reducing possible long-term coexistence. Other laboratory experiments were carried out to identify possible mechanisms that would increase the chance of coexistence. To depict mechanisms underpinning the coexistence observed in greenhouse tomato, we documented: 1) the resource partitioning (*T. absoluta* eggs and larvae) along the plant, and 2) the ability of the parasitoid to avoid plants bearing predators (i.e. choice and non-choice assays). The parasitoid was not able to avoid predator cues, and results on resource partitioning proved to be inconclusive. Therefore coexistence of the two natural enemies may result from differences in their life history traits (e.g. Bonsall et al. 2002, 2004).

Our study suggests that efficient biological control programs targeting a given pest can be based on joint inoculative releases of two different natural enemies. We provide promising results for the development of biological control programs against *T. absoluta* that rely on an endemic larval ectoparasitoid.

## References

Bogran C. E., K. M. Heinz, and M. A. Ciomperlik 2002. Interspecific competition among insect parasitoids: field experiments with whiteflies as hosts in cotton. Ecology 83: 653–668.

Bonsall, M. B., M. P. Hassell, and G. Asefa. 2002. Ecological trade-offs, resource partitioning, and coexistence in a host-parasitoid assemblage. Ecology 83: 925–934.

Bonsall, M. B., V. A. A. Jansen, and M. P. Hassell. 2004. Life history trade-offs assemble ecological guilds. Science 306: 111–114.

Castañé C., O. Alomar, M. Goula, and R. Gabarra. 2004. Colonization of tomato greenhouses by the predatory mirid bugs *Macrolophus caliginosus* and *Dicyphus tamaninii*. Biol. Control 30: 591–597.

Chailleux, A., D. Desneux, J. Seguret, H. Do Thi Khanh, P. Maignet, and E. Tabone. 2012. Assessing European Egg Parasitoids as a Mean of Controlling the Invasive South American Tomato Pinworm *Tuta absoluta*. PloS one 7: e48068.

Chailleux A., E. Mohl, M. Teixeira-Alves, G. J. Messelink, and N. Desneux. 2013. Indirect interactions in agro-ecosystems: potential for optimizing Integrated Pest Management. Pest Manag. Sci. 69: .

Chase, J. M., and M. A. Leibold. 2003. Ecological Niches: Linking Classical and Contemporary Approaches. University of Chicago Press, Chicago, IL, USA.

Desneux, N., E. Wajnberg, K. A. G. Wyckhuys, G. Burgio, S. Arpaia, C. A. Narvaez-Vasquez, J. Gonzalez-Cabrera, D. C. Ruescas, E. Tabone, J. Frandon, J. Pizzol, C. Poncet, T. Cabello, and A. Urbaneja. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. J. Pest Sci. 83: 197–215.

Desneux N., M. G. Luna, T. Guillemaud, and A. Urbaneja. 2011. The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond – the new threat to tomato world production. J. Pest Sci. 84: 403–408.

Finke, D. L., and W. E. Snyder. 2008. Niche partitioning increases resource exploitation by diverse communities. Science 321: 1488–1490.

Gause, G. F. 1934. The struggle for existence. Williams & Wilkins, Baltimore, Maryland, USA.

Lykouressis D. P., D. C. Perdikis, and A. D. Konstantinou. 2009. Predation rates of *Macrolophus pygmaeus* (Hemiptera: Miridae) on different densities of eggs and nymphal instars of the greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). Entomol. Gen. 32: 105–112.

Messelink, G. J., C. M. J. Bloemhard, J. A. Cortes, M. W. Sabelis, and A. Janssen. 2011. Hyperpredation by generalist predatory mites disrupts biological control of aphids by the aphidophagous gall midge *Aphidoletes aphidimyza*. Biol. Control 57: 246–252.

Pimentel, D. 1991. Diversification of biological-control strategies in agriculture. Crop Prot. 10: 243–253.

Pimentel, D. 2005. Environmental and economic costs of the application of pesticides primarily in the United States. Environ. Develop. Sustain. 7: 229–252.

Pilkington L. J., G. Messelink, J. C. van Lenteren, and K. Le Mottee. 2010. Protected Biological Control - Biological pest management in the greenhouse industry. Biol. Control 52: 216–220.

Rosenheim, J. A., H. K. Kaya, L. E. Ehler, J. J. Marois, and B. A. Jaffee. 1995. Intraguild predation among biological-control agents - theory and evidence. Biol. Control 5: 303–335.

Urbaneja, A., J. Gonzalez-Cabrera, J. Arno, and R. Gabarra. 2012. Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. Pest Manag. Sci. 68: 1215–1222.

Zappala, L., U. Bernardo, A. Biondi, A. Cocco, S. Deliperi, G. Delrio, M. Giorgini, P. Pedata, C. Rapisarda, G. Tropea Garzia, and G. Siscaro. 2012. Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in Southern Italy. Bull. Insectol. 65: 51–61.

# Evaluation of native larval parasitoids as BC agents against *Tuta absoluta* in Argentina

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The tomato moth, *Tuta absoluta* (Lepidoptera: Gelechiidae), is a key tomato pest originated from South America. After its introduction into Europe in 2006, it spread across Afro-Euroasia, threatening major tomato producing countries, as India and China. Although it has not been reported in North America yet, it is considered as an emergency pest (Desneux et al. 2011). In Argentina, *T. absoluta* management is mainly based on preventive weekly or bi-weekly applications using about 16 different neurotoxic insecticides (Sánchez et al. 2009). Chemical control costs can reach up to 38% ($175 US per ha per crop cycle) of total sanitary costs.

After hatching, young *T. absoluta* larvae enter into tomato fruits, leaves or stems, on which they feed and develop, thus creating conspicuous mines. Potato, eggplant, tobacco and wild solanaceous species are also reported as hosts of *T. absoluta* (Pereyra and Sánchez 2006).

Biological control of *T. absoluta* is regarded as feasible because there are over 50 species of predators and parasitoids reported throughout the actual pest distribution (Luna et al. 2012). Indeed, *T. absoluta* biological control is commercially available in some countries in Europe and South America by means of augmentative releases of egg and larval parasitoids, and hemipteran predators. In Argentina, despite an increasing interest by producers, biological control remains as an agronomic tool to be developed for this pest (Luna et al. 2007; Pereyra et al. 2010).

Our studies aimed at promoting *T. absoluta* biological control by using entomophagous insects naturally occurring in the local tomato agro-ecosystem. by bringing producers an efficient, low-cost and environmentally safe pest management. We present results on: 1) life history traits of two main *T. absoluta* larval parasitoid wasps, the endoparasitoid *Pseudapanteles dignus* (Hymenoptera: Braconidae) and the ectoparasitoid *Dineulophus phtorimaeae* (Hymenoptera: Eulophidae), 2) their patterns of parasitism in the field, 3) preliminary evaluations of the effectiveness of *P. dignus* inoculative releases on *T. absoluta* control in an experimental tomato greenhouse, and 4) exploration of *P. dignus* and *T. absoluta* alternative hosts (other gelechiids, and cultivated vs. natural vegetation) as baseline information to determine the risks of implementing tomato leafminer biological control. Research was conducted in commercial tomato farms of Northern Buenos Aires province, Argentina. Collaborative work was also done along with INTA.

## References

Desneux, N., M. G. Luna, T. Guillemaud, and A. Urbaneja. 2011. The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. J. Pest Sci. 84: 403–408.

Luna, M. G., N. E. Sanchez, and P. C.Pereyra. 2007: Parasitism of *Tuta absoluta* (Lepidoptera, Gelechiidae) by *Pseudapanteles dignus* (Hymenoptera, Braconidae) under laboratory conditions. Environ. Entomol. 36: 887–893.

Luna, M. G., N. E. Sánchez, P. C. Pereyra, E. Nieves, V. Savino, E. Luft, E, Virla, and S. Speranza. 2012. Biological control of *Tuta absoluta* in Argentina and Italy: evaluation of indigenous insects as natural enemies. EPPO Bull. 42: 260–267.

Pereyra P. C., and N. E. Sánchez. 2006. Effect of two solanaceous host plants on development and population parameters of the tomato leaf miner, *Tuta absoluta*

(Meyrick) (Lepidoptera, Gelechiidae). Neotrop. Entomol. 35: 671–676.

Pereyra, P. C., M.G. Luna, E. Nieves, and N. E. Sánchez. 2010. Spatial parasitism patterns of two parasitoids of *Tuta absoluta* (Lepidoptera: Gelechiidae): the ectoparasitoid *Dineulophus phtorimaeae* and the endoparasitoid *Pseudapanteles dignus* (Hymenoptera: Eulophidae, Braconidae), in tomato crops. Joint IOBC – Nearctic and Neotropic Regional Sections Conference Biocontrol in the Americas May 11-13, 2010. Ontario, Canada.

Sanchez, N. E., P. C. Pereyra, and M. G. Luna. 2009. Spatial patterns of parasitism of the solitary parasitoid *Pseudapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae) on the tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Environ. Entomol. 38: 365–374.

# Implementation of a biological control program for *Tuta absoluta* in greenhouse with the parasitoid *Apanteles gelechiidivoris* in Colombia

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*Tuta absoluta* (Lepidoptera: Gelechiidae) (tomato leafminer) is a major pest of tomatoes (EPPO 2005; Follett 2009, Harizanova et al. 2009). This pest has also been reported on *Lycopersicon hirsutum, Solanum lyratu,* potato (*Solanum tuberosum*), eggplants (*Solanum melongena*)*,* common bean (*Phaseolus vulgaris*)*,* sweet pepper (*Capsicum* spp.) and several wild solanaceous species such as *Solanum nigrum*, *Solanum elaeagnifolium*, *Solanum puberulum*, *Datura stramonium, Datura ferox* and *Nicotiana glauca* (EPPO 2005). In Colombia, it is one of the most important pests of greenhouse tomato crops. The control of *T. absoluta* relies principally on the use of chemical insecticides (Escobar and Lee 2001).

In search of alternative and compatible strategies for the management of *T. absoluta* in Colombia, the Military University "Nueva Granada" has developed research projects to generate the information needed for support the implementation of an IPM program for the pest, in which ethological and biological control are central aspects.

In this context, researches were made in order to determine 1) Biological aspects of the parasite *Apanteles gelechiidivoris* (Hymenoptera: Braconidae), notably its potential, 2) The appropiate timing for releases of *A. gelechiidivoris* according to the infestation level in commercial crops, and 3) Development of a massive rearing system having the capacity to supply the natural enemy in the quantities required, and in a continuous way.

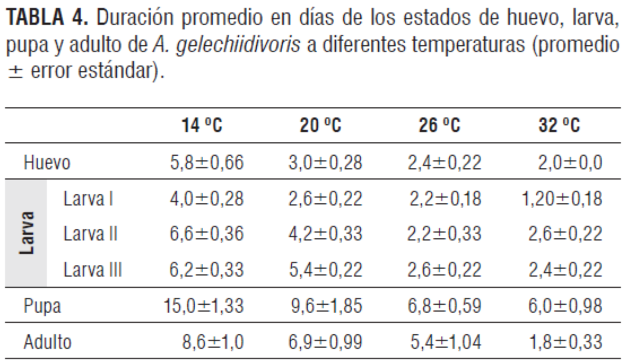


**TABLA 4.** Duración promedio en días de los estados de huevo, larva, pupa y adulto de *A. gelechiidivoris* a diferentes temperaturas (promedio ± error estándar).

| | | 14 ºC | 20 ºC | 26 ºC | 32 ºC |
|---|---|---|---|---|---|
| Huevo | | 5,8±0,66 | 3,0±0,28 | 2,4±0,22 | 2,0±0,0 |
| Larva | Larva I | 4,0±0,28 | 2,6±0,22 | 2,2±0,18 | 1,20±0,18 |
| | Larva II | 6,6±0,36 | 4,2±0,33 | 2,2±0,33 | 2,6±0,22 |
| | Larva III | 6,2±0,33 | 5,4±0,22 | 2,6±0,22 | 2,4±0,22 |
| Pupa | | 15,0±1,33 | 9,6±1,85 | 6,8±0,59 | 6,0±0,98 |
| Adulto | | 8,6±1,0 | 6,9±0,99 | 5,4±1,04 | 1,8±0,33 |

*A. gelechiidivoris* has four stages: e.g. egg, larva (with three instars), pupa and adult. Its developmental time is function of temperature, from two days at 32°C to 9 days at 14°C.

Adults were obtained in the range between 14°C-32°C and the highest female proportion was observed at 20°C.

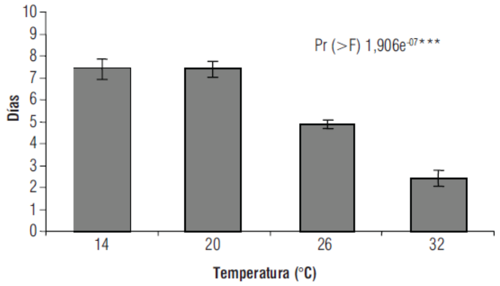


**FIGURA 4.** Longevidad de adultos de *A. gelechiidivoris* en función de diferentes temperaturas (promedio ± error estándar).

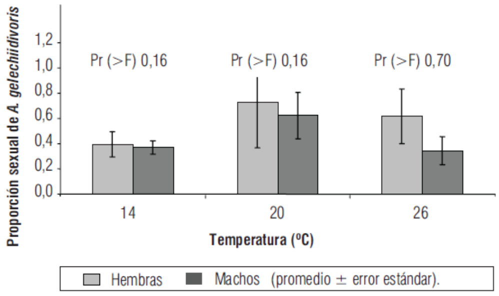


**FIGURA 3.** Proporción de machos y hembras de *A. gelechiidivoris* emergidos a diferentes temperaturas.

Figure 1. Life cycle and parasitic capacity of *Apanteles gelechiidivoris* (Hymenoptera: Braconidae) for the control of *T. absoluta* (See Bajonero et al. 2008 and Escobar et al. 2004)

Functional response curves at different temperatures indicated that the highest parasitic capacity is obtained at 20°C, corresponding to 12 larvae of third instar. In combined tests with sex pheromone-baited traps, releases of *A. gelechiidivoris* on *T. absoluta* in proportion 1:12 (obtained from the functional response) achieved 66% parasitism on tomatoes grown in commercial greenhouse (Bajonero 2011).

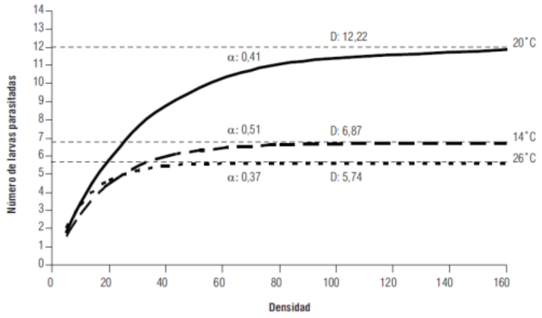


**FIGURA 2.** Respuesta funcional de *A. gelechiidivoris* sobre larvas de *T. absoluta* a diferentes temperaturas: 14 ºC (——), 20 ºC (— —) y 26 ºC (- - -).

Figure 2. Effect of densities of *T. absoluta* on growth and development of greenhouse tomato plants (see Cely et al. 2011)

The effect of five densities of females/plant (2, 4, 6, 8, 10) was evaluated. Three phases are clearly noticeable in the foliar damage curve: 1) Damage increase at growing rate, where the consumption of the leaf tissue is caused by first instar larvae, 2) Damage increase at linear maximum rate, especially by the feeding of larvae corresponding to 2d and 3d instars, and 3) Stabilization of foliar damage. The total number of fruit and healthy fruit produced per plant in the first and second clusters reduces as infestation density increases. The greatest amount of perforated fruit was obtained in infestation densities of 8 and 10 females/plant, which is equivalent to densities over 100 larvae per plant with 55 and 100% damage, respectively.

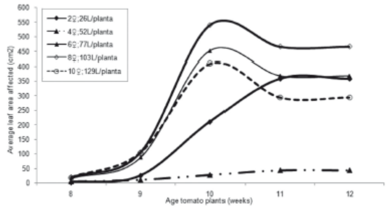
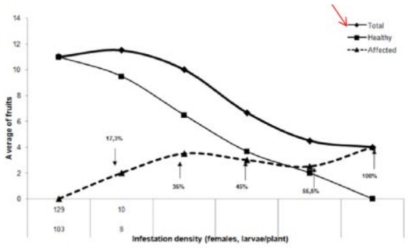


FIGURE 2. Average total leaf area affected by *T. absoluta* in greenhouse tomato plants between eight to twelve weeks of age.



Average healthy and affected fruits by *T. absoluta* for the first two clusters in greenhouse tomato plants.

Figure 3. Rearing system of *A. gelechiidivoris* for continuous supply of the natural enemy (see Morales 2011)

A rearing system that maximizes the proportion of females of the parasitoid was developed, using as natural diet third instar larvae of *T. absoluta* grown on tomato plants.

## References

Bajonero, J., N. Córdoba, F. Cantor, D. Rodríguez, and J. R. Cure. 2008. Biología y ciclo reproductivo de *Apanteles gelechiidivoris* (Hymenoptera: Braconidae), parasitoide de *Tuta absoluta* (Lepidoptera: Gelechiidae). Agron. Colomb. 26: 417–426.

Bajonero, J. 2011. Evaluación de trampas de feromona sexual y *Apanteles gelechiidivoris* para el manejo de *Tuta absoluta* (Lepidoptera: Gelechiidae) en un cultivo comercial de tomate bajo invernadero. Tesis de Magister Biología Aplicada, Universidad Militar Nueva Granada, Colombia. 66p.

Cely, L., F. Cantor, and D. Rodríguez. 2011. Determinación de niveles de daño ocasionados por diferentes densidades de población de *Tuta absoluta* (Lepidoptera: Gelechiidae) en tomate bajo invernadero. Agron. Colomb. 28:37–146.

Escobar, H., and R. Lee. 2001. Producción de tomate bajo invernadero. Fundación Universidad de Bogotá Jorge Tadeo Lozano, 70p.

Escobar, A., F. Cantor, and J. R. Cure. 2004. Contribución al conocimiento de la biología de *Apanteles gelechiidivoris* (Hymenoptera: Braconidae). Revista Facultad de Ciencias, Universidad Militar Nueva Granada, Bogotá-Colombia. 1: 71–74.

EPPO- European and Mediterranean Plant Protection Organization (2005). Data sheets on quarantine pests - *Tuta absoluta*. OEPP/EPPO Bull. 35: 434–435.

Follet,. P A. 2009. Generic Radiation Quarantine Treatments: The Next Steps. J. Econ. Entomol. 102: 1399–1406.

Harizanova, V., A. Stoeva, and M. Mohamedova. 2009. Tomato leaf miner, *Tuta absoluta* (Povolny) (Lepidoptera: gelechiidae) – first record in Bulgaria. Agric. Sci. Technol. 1: 95–98.

Morales, J. 2011. Estandarización de la cría masiva de *Apanteles gelechiidivoris* (Hymenoptera: Braconidae) para el control de *Tuta absoluta* (Lepidoptera: Gelechiidae). Tesis de Magister Biología Aplicada, Universidad Militar Nueva Granada, Colombia. 74p.

# Session 18: Compatibility of Transgenic Insecticidal Crops with Biological Control

## Using Bt-resistant hosts to remove prey quality effects when investigating potential effects of Bt plants on natural enemies

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*Introduction:* A long-term goal of our work is to provide science-based information to regulatory agencies on the environmental impacts of insect resistant genetically-modified (IRGM) plants on nontarget organisms, especially beneficial arthropods attacking important crop pests. Without such information, regulatory agencies cannot ensure the environmental safety of the products they regulate. Recent examples of studies showing negative environmental effects of IRGM plants, although criticized for their design and methods, have had tremendous negative effects on regulatory systems (Shelton et al. 2009) and led to confusion in the scientific community. A common problem in studies on non-target beneficial arthropods is that potential effects of prey-quality are not taken into account. This usually results when predators or parasitoids feed on susceptible hosts that have ingested Cry proteins. The outcome is that as the host suffers, so does the natural enemy, leading some to suggest there is a direct effect of the Cry protein on the natural enemy. Such a case is the review by Lövei et al. (2009) who erroneously suggested negative effects on natural enemies by Bt proteins. One way of overcoming the effect of prey-quality is to feed non-susceptible hosts the Cry protein and then allow the predator or parasitoid to feed on this uncompromized host. However, this has limitations because most of the appropriate systems (Cry protein-host insect-natural enemy) are not available because the host is either susceptible to the Cry protein or the natural enemy of interest does not attack the particular host. Ideally, study systems should consist of once-susceptible hosts that have developed resistance to the Cry protein and natural enemies that typically feed on the host in the field. Published reports indicate that such tritrophic systems have been rarely used (Romeis et al. 2011).

*Our approach:* The main Bt proteins commercially used to control Lepidoptera are Cry1Ac, Cry2Ab and Cry1F. We have strains of insects that have evolved resistance to each of these proteins: cabbage looper (CL), *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), which is a pest of cotton and is resistant to Cry1Ac and Cry2Ab; Fall armyworm (FAW), *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), which is a pest of maize and resistant to Cry1F; and European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), which is a pest of maize and is resistant to Cry1F. We have conducted a series of studies in which we fed these resistant insects plant material expressing the Bt proteins, and then fed them to their respective suite of natural enemies. We then assessed biological parameters such as development time, mortality, weight and oviposition of the natural enemies (parasitoids and predators) that had preyed on hosts that had ingested the Bt proteins. The controls were the same pest insect but which had fed on non-Bt plants. To confirm that the natural enemies had come into contact with the Cry proteins when they had preyed on the resistant hosts, we examined them for the presence of bioactive Cry proteins. To provide information on potential effects of Cry proteins across a breadth of natural enemies, we are examining a wide range of natural enemies from diverse families in several insect orders including Coleoptera, Hemiptera, Hymenoptera, Neuroptera, Diptera and Dermaptera.

*Results to date*: Using Cry1F-resistant FAW larvae as prey, we found that the fitness parameters of the ladybird beetle, *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae), did not differ over two generations (Tian et al. 2012). Duration of larval and pupal stages, adult weight and female fecundity of *C. maculata* were not different when they were fed resistant FAW larvae reared on either Bt or control maize leaves during both generations. ELISA and insect-sensitive bioassays showed *C. maculata* were exposed to bioactive Cry1F protein. The insecticidal protein had no effect on *C. maculata* larvae, even though larvae contained 20-32 ng of Cry1F/g of fresh weight. Similarly, lack of differences in fitness parameters have also been observed when the lacewing, *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae), fed on Cry1F-resistant FAW and Cry1Ac and Cry2Ab-resistant CL; when the parasitoid, *Copidosoma floridanum* (Ashmead) (Hymenoptera: Encyrtidae), fed on Cry1Ac- and Cry2Ab-resistant CL; when the parasitoid, *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) fed on Cry1Ac and Cry2Ab-resistant CL, and; when the predators, *Geocoris punctipes* (Say) (Hemiptera: Geocoridae) and *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), fed on Cry1Ac and Cry2Ab-resistant CL. Additional studies are underway and will be reported at the meeting.

*Conclusion:* Our results to date confirm the safety of Cry1Ab, Cry2Ab and Cry1F to common natural enemies of the lepidopteran pests of maize and cotton. These results provide additional evidence of the safety of these proteins beyond what was presented in the reviews and meta-analyses by Romeis et al. (2006), Wolfenbarger et al. (2008), Naranjo (2009), and Duan et al. (2010).

## References

Duan, J. J., J. G. Lundgren, S. E. Naranjo, and M. Marvier. 2010. Extrapolating non-target risk of Bt crops from laboratory to field. Biol.Letters 6: 74–77.

Lövei, G. L., D. A. Andow, and S. Arpaia. 2009. Transgenic insecticidal crops and natural enemies: A detailed review of laboratory studies. Environ. Entomol. 38: 293–306.

Naranjo, S. E. 2009. Impacts of *Bt* crops on non-target organisms and insecticide use patterns. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 4: No.011. doi: 10.1079/PAVSNNR20094011.

Romeis, J., M. Meissle, and F. Bigler. 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nat. Biotechnol. 24: 63–71.

Romeis, J., R. L. Hellmich, M. P. Candolfi, K. Carstens, A. De Schrijver, A. M. R. Gatehouse, R. A. Herman, J. E. Huesing, M. A. McLean, A. Raybould, A. M. Shelton, and A. Waggoner. 2011. Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. Transgenic Res. 20: 1–22.

Shelton, A. M., S. E. Naranjo, J. Romeis, R. L. Hellmich, J. D. Wolt, B. A. Federici, R. Albajes, F. Bigler, E. P. J. Burgess, G. P. Dively, A. M. R. Gatehouse, L. A. Malone, R. Roush, M. Sears, and F. Sehnal. 2009. Setting the record straight: a rebuttal to an erroneous analysis on transgenic insecticidal crops and natural enemies. Transgenic Res. 18: 317–322.

Tian, J., H. L. Collins, J. Romeis, S. E. Naranjo, R. L. Hellmich, and A. M. Shelton. 2012. Using field-evolved resistance to Cry1F maize in a lepidopteran pest to demonstrate no adverse effects of Cry1F on one of its major predators. Transgenic Res. doi 10.1007/s11248-012-9604-4.

Wolfenbarger, L. L., S. E. Naranjo, J. G. Lundgren, R. J. Bitzer, and L. S. Watrud. 2008. Bt crops effects on functional guilds of non-target arthropods: A meta-analysis. PLoS ONE 3(5): e2118. doi/10.1371/journal.pone.0002118.

# Spiders entangled in environmental risk assessment of genetically engineered crops

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*Introduction:* Before new genetically engineered (GE) crops can be grown commercially, potential risks to the environment need to be assessed. Risk assessment ensures that desired ecological functions, such as biological control, pollination, and decomposition are not harmed. Insecticidal GE crops currently on the market express proteins from the bacterium *Bacillus thuringiensis* Berliner (Bacillaceae) (Bt) that are toxic to the order of Lepidoptera or the family of Chrysomelidae (Coleoptera). Those crops have replaced broad spectrum insecticides by controlling the most damaging pests. Other herbivores, not targeted by the Bt crop, remain unaffected whereas broad spectrum insecticides used to control them as well. In Bt crops, natural enemies are thus vital to suppress secondary pests below the economic threshold. In an Integrated Pest Management (IPM) context, this is desirable, because a responsive measure, i.e. insecticide application, is replaced by two preventive measures, i.e. host plant resistance and conservation of natural enemies (Meissle et al. 2011).

The predator community in major European crops is dominated by ground-, rove-, and ladybeetles (Coleoptera), followed by spiders (Araneae) (Meissle et al. 2012). Spiders have long been recognized as abundant invertebrate predators in agroecosystems that play an important role in biological pest control in many crops (Marc et al. 1999, Symondson et al. 2002, Meissle and Lang 2005). In this paper, the spider fauna recorded in European crops is presented in the context of environmental risk assessment. Furthermore, exposure of spiders to Bt proteins is discussed and our knowledge on effects of Bt proteins on spiders in laboratory and field experiments is summarized.

*Non-target risk assessment of GE crops:* Knowledge on the receiving environment, i.e. the arthropod community inhabiting the GE crop in the region where the crop will be grown is important to formulate relevant risk hypothesis to be tested in environmental risk assessment. A typical risk hypothesis is that “the insecticidal protein is not toxic to valued non-target organisms at the concentration present in the field”. The risk hypotheses are then addressed in the analysis phase of the risk assessment following a tiered approach. For insecticidal proteins, early-tier testing is conducted under worst-case exposure conditions in the laboratory. Surrogate test species are selected that are most likely to reveal an adverse effect (Romeis et al. 2013). More complex and realistic higher-tier assessments under semi-field or field conditions are only necessary when adverse effects indicating potentially unacceptable risk have been detected in early tier testing or when unacceptable uncertainties remain.

*Spiders in European field crops:* A database on arthropods in European field crops, commissioned by the European Food Safety Authority (EFSA) (Meissle et al. 2012), was filtered for spider species in crop fields. More than 60% of all spider records were obtained from maize, followed by oilseed rape, beet, potato (9-15% each), and soybean and cotton (2% of records). All records were sorted into ground-dwelling species and plant-dwelling species according to the sampling methods applied. For ground-dwelling spiders, 1163 records of spiders in European crops were available from pitfall traps, emergence traps, quadrat sampling, visual collections, and soil and litter samples. In contrast, only 158 records of plant-dwelling spiders were available from aspirator sampling, beatsheet sampling, visual counts and collections, plant removal, stem traps, and sweep net sampling.

The most collected ground-dwelling spider families were Linyphiidae (49% of all records) and Lycosidae (17%). Gnaphosidae, Theridiidae, Tetragnathidae, Thomisidae, and Salticidae represented 3-8% of the total records and further 16 families were recorded (1% or less). The most collected ground-dwelling Linyphiidae were *Oedothorax apicatus* (Blackwall), *Meioneta rurestris* (C. L. Koch), *Erigone atra* Blackwall, and *Erigone dentipalpis* (Wider). Lycosidae were represented by different *Pardosa* spp, such as *P. agrestis* (Westring), *P. palustris* (L.), *P. prativaga* (L. Koch), and *P. amentata* (Clerck) and by *Trochosa ruricola* (De Geer).

The community of plant-dwelling spiders was dominated by Linyphiidae (36%), Araneidae (19%), and Theridiidae (19%). Thomididae, Tetragnathidae, Pisauridae, and Lycosidae represented 3-7% of the total records and further 6 families were recorded (2% or less). The most collected species were *Phylloneta impressa* (L. Koch) (Theridiidae), *Aculepeira ceropegia* (Walckenaer) (Araneidae), *Pachygnatha degeeri* Sundevall (Tetragnathidae), and *Pisaura mirabilis* (Clerck) (Pisauridae). The plant-dwelling Linyphiidae were dominated by the same species as the ground-dwelling Linyphiidae.

If early tier risk assessment of new GE crops cannot rule out a potential hazard for spiders, the previously mentioned species might be suitable representatives of the spider fauna that could be used for semi-field or field experiments. Those species are widespread throughout Europe and occur with high abundance, which allows minimizing sampling effort by maintaining relatively high statistical power in field studies.

*Exposure of spiders to plant-expressed Bt proteins:* In Bt crops, spiders can be exposed when feeding on prey which has consumed Bt proteins. Exposure of spiders via prey depends on the actual amount of the insecticidal protein contained in the prey species. Herbivores feeding on Bt plant tissue contain relatively high concentrations of Cry protein (Harwood et al. 2005, Meissle and Romeis 2009), while phloem-feeding species contain no or at best traces of Cry protein, which is not transported in the phloem (Romeis and Meissle 2011). In predatory species, concentrations are generally lower than in the tissue-feeding herbivores. Because most spiders consume a broad range of prey species with different feeding habits and thus different Bt protein contents, environmental exposure concentrations of spiders in a Bt crop are highly variable. The average exposure, however, is considerably below the concentration in leaf tissue, because the Cry proteins get diluted when transferred to higher trophic levels (Meissle and Romeis 2012).

Spiders may also feed on pollen of Bt maize or cotton. Pollen availability in those crops, however, is limited. Cry concentrations in pollen are often low, and uptake by spiders seems to be low even in plants with relatively high expression levels in pollen. This indicates that exposure of spiders to Cry proteins via pollen is less relevant than exposure via prey.

*Effects of Bt proteins in laboratory experiments:* No negative effects of Bt maize were observed on *P. impressa* (Meissle and Romeis 2009) and *Araneus diadematus* Clerck (Araneidae) (Ludy and Lang 2006) when spiders consumed pollen or Bt-maize-fed prey. Similarly, Bt proteins expressed in rice had no direct effect on *Ummeliata insecticeps* (Bösenberg & Strand) (Linyphiidae) (Tian et al. 2010), *Pardosa pseudoannulata* (Bösenberg & Strand) (Lycosidae) (Tian et al. 2012), and *Pirata subpiraticus* (Bösenberg & Strand) (Lycosidae) (Chen et al. 2009) when prey from Bt rice was fed to the spiders. The species selected for those laboratory tests in Europe and Asia belong to the families Theridiidae, Araneidae, Linyphiidae, and Lycosidae.

Interestingly, those families also represent the most collected spiders in European field crops. From a risk assessment perspective, we can thus conclude that the chosen species in the available laboratory studies can serve as surrogates also for the spider fauna in European field crops. They might also represent major spider families in field crops in other world areas (Nyffeler and Sunderland 2003).

*Effects of Bt crops in field experiments:* Numerous field studies confirmed that spiders are not adversely affected by plant-expressed Bt proteins. Altogether 60 studies that contain spider data from maize, cotton, potato, rice, and eggplant were subjected to meta-analysis by Peterson et al. (2011). No effect of Bt maize on the abundance of foliage and ground-dwelling spiders compared to untreated conventional maize was evident, while Bt maize harboured more spiders than insecticide treated conventional maize. No difference in spider abundance was evident between Bt and untreated non-Bt cotton for ground-dwelling spiders. The abundance of foliar spiders in Bt cotton was marginally lower relative to untreated non-Bt fields, but the effect size was small compared to the positive effect of Bt cotton versus insecticide treated conventional cotton. Similarly, field studies on potato, rice, and eggplant reported no deleterious effects on spiders (Peterson et al. 2011, Tian et al. 2010). Those findings are in line with reviews (Romeis et al. 2006) and meta-analyses (Naranjo 2009) on a broad range of beneficial arthropods, which confirmed the narrow spectrum of activity of Cry proteins expressed in today's Bt crops.

*Conclusions:* Spiders are abundant and important predators in field crops. For Europe, families with the highest number of collection records include Linyphiidae, Lycosidae, Theridiidae, and Araneidae. Laboratory studies with representative species from those families and a number of field studies with commercialized and experimental Bt crops demonstrated that Bt crops pose a negligible risk for spiders.

## References

Chen, M., G. Y. Ye, Z. C. Liu, Q. Fang, C. Hu, Y. F. Peng, and A. M. Shelton. 2009. Analysis of Cry1Ab toxin bioaccumulation in a food chain of Bt rice, an herbivore and a predator. Ecotoxicology 18: 230–238.

Harwood, J. E., W. G. Wallin, and J. J. Obrycki. 2005. Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Mol. Ecol. 14: 2815–2823.

Ludy, C., and A. Lang. 2006. Bt maize pollen exposure and impact on the garden spider, *Araneus diadematus*. Entomol. Exp. Appl. 118: 145–156.

Marc, P., A. Canard, and F. Ysnel. 1999. Spiders (Araneae) useful for pest limitation and bioindication. Agric. Ecosys. Environ. 74: 229–273.

Meissle, M., and A. Lang. 2005. Comparing methods to evaluate the effects of Bt maize and insecticide on spider assemblages. Agric. Ecosys. Environ. 107: 359–370.

Meissle, M., and J. Romeis. 2009. The web-building spider *Theridion impressum* (Araneae: Theridiidae) is not adversely affected by Bt maize resistant to corn rootworms. Plant Biotechnol. J. 7: 645–656.

Meissle, M., and J. Romeis. 2012. No accumulation of Bt protein in *Phylloneta impressa* (Araneae: Theridiidae) and prey arthropods in Bt maize. Environ. Entomol. 41: 1037–1042.

Meissle, M., J. Romeis, and F. Bigler. 2011. Bt maize and integrated pest management – a European perspective. Pest Manag. Sci. 67: 1049–1058.

Meissle, M, F. Álvarez-Alfageme, L. A. Malone, and J. Romeis. 2012. Establishing a database of bio-ecological information on non-target arthropod species to support the environmental risk assessment of genetically modified crops in the EU.

Supporting Publications 2012:EN-334. European Food Safety Authority (EFSA), Parma, Italy. Available online: http://www.efsa.europa.eu/en/supporting/pub/334e.htm

Naranjo, S. E. 2009. Impacts of Bt crops on non-target invertebrates and insecticide use patterns. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 4: No. 011.

Nyffeler, M. and K. D. Sunderland. 2003. Composition, abundance and pest control potential of spider communities in agroecosystems: a comparison of European and US studies. Agric. Ecosys. Environ. 95: 579–612.

Peterson, J. A., J. G. Lundgren, and J. D. Harwood. 2011. Interactions of transgenic *Bacillus thuringiensis* insecticidal crops with spiders (Araneae). J. Arachnol. 39: 1–21.

Romeis, J., M. Meissle, and F. Bigler. 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nature Biotechnol. 24: 63–71.

Romeis, J. and M. Meissle. 2011. Non-target risk assessment of Bt crops – Cry protein uptake by aphids. J. Appl. Entomol. 135: 1–6.

Romeis, J., A. Raybould, F. Bigler, M. P. Gandolfi, R. L. Hellmich, J. E. Huesing, and A. M. Shelton. 2013. Deriving criteria to select arthropod species for laboratory tests to assess the ecological risks from cultivating arthropod-resistant genetically engineered crops. Chemosphere 90: 901–909.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Ann. Rev. Entomol. 47: 561–594.

Tian, J. C., Y. Chen, Z. L. Li, K. Li, M. Chen, Y. F. Peng, C. Hu, A. M. Shelton, and G. Y. Ye. 2012. Transgenic Cry1Ab rice does not impact ecological fitness and predation of a generalist spider. PLoS ONE 7: e35164.

Tian, J. C., Z. C. Liu, M. Chen, Y. Chen, X. X. Chen, Y. F. Peng, C. Hu, G. Y. Ye. 2010. Laboratory and field assessments of prey-mediated effects of transgenic Bt rice on *Ummeliata insecticeps* (Araneida: Linyphiidae). Environ. Entomol. 39: 1369–1377.

# Effects of *Bt* crops on arthropod natural enemies: A global synthesis

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*Introduction:* The global adoption of transgenic crops producing the insecticidal proteins from *Bacillus thuringiensis* Berliner (Bacillaceae), (*Bt*) continues to grow with 66 M hectares of *Bt* crops grown in a total of 25 countries in 2011 (James 2011). Unintended environmental effects from the technology continue to be of concern, with one of the key issues being effects on valued non-target organisms. A large number of non-target studies have been completed over the past 15-20 years in support of risk assessment in *Bt* crops. In late 2008 Naranjo (2009) cataloged over 360 original research articles that have studied the effects of *Bt* crops on non-target invertebrates. These include studies of 9 crop plants and dozens of *Bt* proteins in a total of 15 countries. Since 2008 the pace of research in this area has diminished only slightly, with a total of over 520 studies as of mid 2012, and the number of participating countries has expanded. A number of reviews and meta-analyses have been conducted on portions of this literature in an attempt to summarize and synthesize general and specific patterns (e.g. Romeis et al. 2006, Marvier et al. 2007, Wolfenbarger et al. 2009, Naranjo 2009). In general, these summaries and analyses have demonstrated that *Bt* crops have negligible or no effect on non-target arthropod abundance in the field or on various measures of their biology in the laboratory. However, many additional studies have been published since the latest reviews and synthetic studies were completed. Here I re-assess the world literature on arthropod natural enemies and quantify non-target effects of *Bt* crops via meta-analyses of both laboratory and field studies. The primary question is: do we now have enough data to conclusively determine the effects of current *Bt* crops on non-target arthropod natural enemies?

*Background and Approach:* Marvier et al. (2007) assembled the original non-target invertebrate database and published the first general meta-analysis based on field studies examining *Bt* cotton and maize. A year later Wolfenbarger et al. (2008) modified the database and performed meta-analyses to examine the effects of *Bt* cotton, maize and potato on the field abundance of ecological functional guilds. Naranjo (2009) then updated the full database with a number of new field and laboratory studies, performed additional meta-analysis of the field studies and also completed the first meta-analyses of laboratory studies. Finally, Duan et al. (2010) performed meta-analyses to examine the relationship between laboratory and field results based on a subset of the database focused on survival. Here, I have again updated the database, but only for natural enemy guilds. This latest database now includes 92 laboratory and 67 field studies on arthropod natural enemies. Many potential studies could not be included due to incomplete data reporting by study authors. This new database covers 1 Phylum, 2 Classes, 13 Orders and 46 Families of predators and 1 Phylum, 1 Class, 3 Orders and 25 Families of parasitoids. For meta-analyses I used Hedge's d, a weighted effect size estimator that is calculated as the difference between an experimental (*Bt*) and control (non-*Bt*) mean response (abundance, survival rate, development time, fecundity, etc.) divided by a pooled standard deviation and corrected for small sample size bias. Fixed, weighted categorical analyses were performed with MetaWin 2.1 (Rosenberg et al. 2000).

*Results:* I first conducted a cumulative meta-analysis on the field studies for two functional guilds, predator and parasitoids (Figure 1). This analysis provides a running snapshot of overall results as new studies became available over time. It clearly shows that while results have become less variable over time, with increased sample size, the conclusion that *Bt* crops do not significantly affect the abundance of non-target natural enemies has been clear from even the earliest studies. The addition of new studies since 2008 has not altered that conclusion.

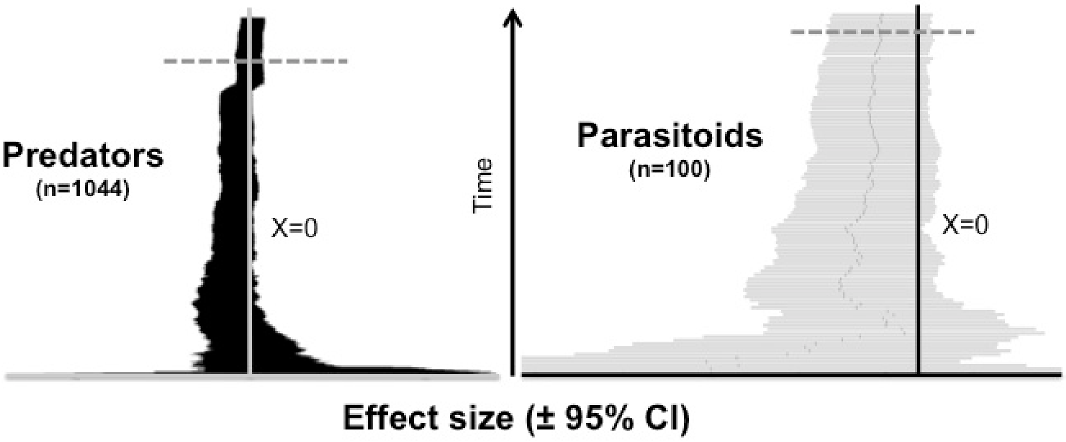

Figure 1. Cumulative meta-analysis of field studies examining abundance of arthropod predators and parasitoids. The analyses show the effect of adding more studies over time on the effect size and its associated 95% confidence interval. The dotted gray line indicates new studies that were added since 2008. Results show that the effect size was never significantly different from zero (=no difference between *Bt* and non-*Bt* treatments) and that conclusion has not changed with the addition of recent studies.

The study database was then parsed by country of origin for these two functional groups. For 11 countries, effect sizes were not significantly different from zero for either group. Studies for predators in the USA actually show a significant positive effect size indicating that abundance was on average slightly higher in *Bt* crops compared with the non-*Bt* control. Parsing by five *Bt* crops (cotton, maize, potato, eggplant, rice), again effects on predators and parasitoids were largely neutral, the only difference being a higher abundance of predators on *Bt* potato. Neutral effects were also seen for predators and parasitoids regardless of the pest target of the *Bt* crop (Lepidoptera or Coleoptera) or the number or type of insecticidal proteins produced by the crop (single or pyramided, Cry or VIP). These results are all consistent with prior meta-analyses (Wolfenbarger et al. 2008, Naranjo 2009) as are the results that the alternative use of insecticides to control the target pest led to large reductions in abundance of natural enemies in non-*Bt* crops.

Laboratory studies have focused on either direct exposure of natural enemies to *Bt* proteins through plant material or spiked artificial diets, or more commonly, through tri-trophic exposures where the prey is fed on *Bt* plants or diets and then provided to the predator or parasitoid. The former exposure pathway has provided unequivocal results: *Bt* proteins do not affect life history characteristics such as development, reproduction or survival. Results from tri-trophic exposure studies have been variable (Figure 2), but careful examination of these studies show that the nature of the prey provided to the natural enemy is critical to interpreting *Bt* effects. Prey that are susceptible to the *Bt* proteins (target pests or related species) are negatively affected by these proteins and this reduces their quality as prey for natural enemies. Results clearly show, especially for parasitoids, that attacking these compromised, or low quality, prey negatively affects life history traits. However, if low quality issues are removed by using non-susceptible prey or prey that are resistant to *Bt* proteins then effects of the *Bt* proteins are either neutral or sometimes even positive (Figure 2). This provides conclusive evidence that it is prey quality and not *Bt* proteins that are associated with negative effects. This also explains why recent results from an analysis by Lövei et al. (2009, but see rebuttal by Shelton et al. 2009) deviate from all other synthetic analyses and reviews – they did not account for prey quality mediated effects.

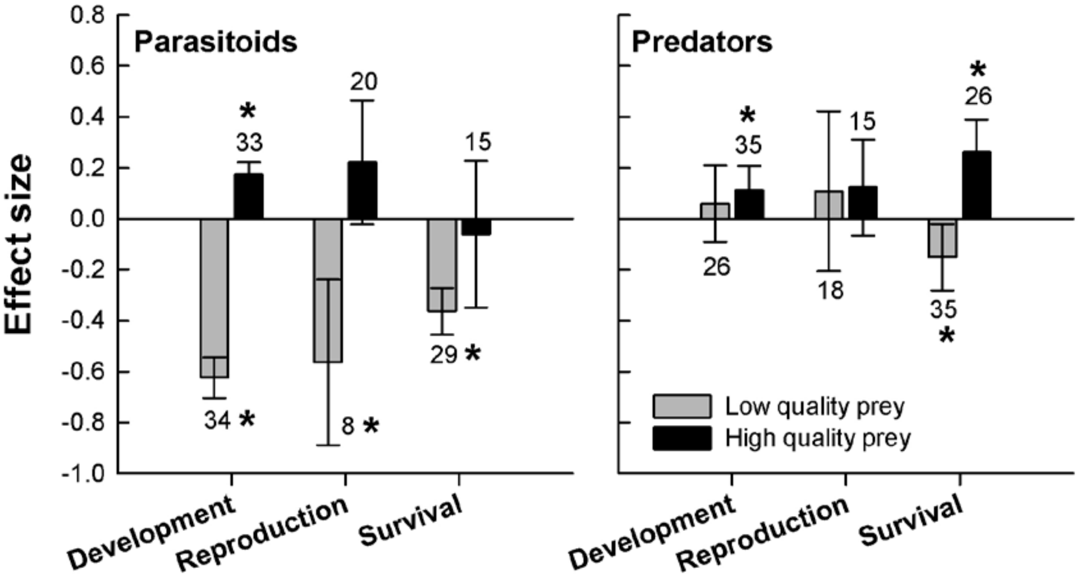

Figure 2. Meta-analyses of studies examining effects of *Bt* proteins on arthropod predators and parasitoids when the natural enemy was exposed to the *Bt* proteins indirectly through their prey. Prey are either susceptible to the *Bt* proteins, and thus compromised (low quality), or unaffected by *Bt* proteins (high quality). Mean effect sizes are plotted so that negative values indicate poorer performance from indirect *Bt* exposure. Error bars are 95% confidence intervals and asterisks indicate the mean effect size is significantly different from zero; numbers are sample size. Modified from Naranjo (2009) to include new studies since 2008.

*Conclusion:* A large number of laboratory and field studies have been conducted to measure the non-target effects of *Bt* crops and several reviews and meta-analyses have synthesizes these data. Here I show that the addition of new studies since 2008 have not changed the conclusion that *Bt* proteins are highly selective and do not negatively affect non-target arthropod natural enemies. For field studies, origin of the study, crop species, or the type or pyramiding of proteins does not influence these results. Laboratory studies clearly show no direct effects of *Bt* protein on life history characteristics of arthropod predators or parasitoids and further show that any indirect negative effects of exposure of natural enemies through their prey are due to prey quality and not to *Bt* proteins. Overall, the analyses support the conclusion that available data indicate a lack of effect of *Bt* proteins on arthropod natural enemies.

## References

Duan, J. J., J. G. Lundgren, S. E. Naranjo, and M. Marvier. 2010. Extrapolating non-target risk of *Bt* crops from laboratory to field. Biol. Lett. 6: 74–77.

James, C. 2011. Global status of commercialized biotech/GM crops: 2011. ISAAA Briefs, No. 43. (http://www.isaaa.org/resources/publications/briefs/43/default.asp)

Lövei, G. L., D. A. Andow, and S. Arpaia. 2009. Transgenic insecticidal crops and natural enemies: A detailed review of laboratory studies. Environ. Entomol. 38: 293–306.

Marvier, M., C. McCreedy, J. Regetz, and P. Kareiva. 2007. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. Science 316: 1475–1477.

Naranjo, S. E. 2009. Impacts of *Bt* crops on non-target organisms and insecticide use patterns. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 4: No. 011 (DOI: 10.1079/PAVSNNR20094011).

Romeis, J., M. Meissle, and F. Bigler. 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nat. Biotechnol. 24: 63–71.

Rosenberg, M. S., D. C. Adams, and J. Gurevitch. 2000. MetaWin Version 2: Statistical Software for Meta-Analysis. Sinauer Associates, Inc, Sunderland, Massachusetts.

Shelton, A. M., S. E. Naranjo, J. Romeis, R. L. Hellmich, J. D. Wolt, B. A. Federici, R. Albajes, F. Bigler, E. P. J. Burgess, G. P. Dively, A. M. R. Gatehouse, L. A. Malone, R. Rousch, M. Sears, and F. Sehnal. 2009. Appropriate analytical methods are necessary to assess non-target effects of insecticidal proteins in GM crops through meta-analysis (response to Andow et al.). Environ. Entomol. 38: 1533–1538.

Wolfenbarger, L. L., S. E. Naranjo, J. G. Lundgren, R. J. Bitzer, and L. S. Watrud. 2008. Bt crops effects on functional guilds of non-target arthropods: A meta-analysis. PLoS One 3:e2118. (DOI: 10.1371/journal.pone.0002118)

# Poster Abstracts

## Session 1:Risk Assessment in Arthropod Biological Control: Where are we?

### *Heringia calcarata* (Diptera: Syrphidae) – a potential biocontrol agent for woolly apple aphid in New Zealand

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Importation of *Heringia calcarata* Loew (Diptera: Syrphidae) into containment in New Zealand was approved in December 2010. Four shipments of eggs and larvae of the hoverfly were subsequently sent from Virginia (USA) to New Zealand between July and October 2012, and the survival and longevity of different life-stages were studied. Insects were shipped in tubes within an expanded polystyrene box with freezer pads to moderate the temperature, and transported by commercial courier or as hand luggage. The journeys took from 3 to 11 days. Although all permits and travel documentation were attached, two shipments were delayed for several days in the USA or in New Zealand by bureaucratic concerns over their commercial value or biosecurity status. Most eggs had hatched on arrival, and larvae were usually transferred immediately to New Zealand-sourced woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Pemphigidae), (WAA). No 2nd instar (or older) *H. calcarata* were lost or died in transit. Residual apple twigs and woolly apple aphids from the USA were destroyed. Larvae were held individually in petri-dishes at 24–26°C and preferably >80% ambient RH, and WAA were added as required. When mature larvae voided their gut contents, they were transferred to clean petri dishes with folded filter paper in which to pupate. Some field collected larvae had been parasitised in Virginia by *Phthorima bidens* Davis (Hymenoptera: Ichneumonidae), which emerged from pupae. Adult hoverflies were held in various sized cages under artificial lighting at 24°C and 65-70%RH or at 26°C and >80%RH. They were supplied with bee pollen from *Apis mellifera* L. (Hymenoptera: Apidae), glucose and fresh flowers to provide floral nectar and pollen ad libitum; and apple shoots with WAA colonies for oviposition. No mating behaviour was observed in any of the cages. Some females laid eggs adjacent to WAA colonies but none was fertile. Adults lived for a mean of 15.4 ±5.5 days (females) and 13.5 ±4.1 days (males). Designing a system for mating *H. calcarata* remains a crucial future step for the development of mass rearing and host-specificity testing in containment.

# Prediction of non-target impacts: can results be generalised between related species?

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Host specificity tests are an essential part of new biological control programs yet these tests are costly, time consuming and the results from tests conducted under quarantine conditions do not always reflect what happens in the natural environment. If a species has been subject to host specificity tests, the test outcomes may act as a guide to predicting test outcomes from another closely related species. For example, *Trichogramma platneri* Nagarkatti (Hymenoptera: Trichogrammatidae) is a generalist parasitoid of Lepidoptera used for inundative biological control that is also able to parasitise eggs of the green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). In Australia there are three trichogrammatid parasitoids available commercially for inundative biological control. These parasitoids represent a gradient of host specificity towards lepidopteran pests: *Trichogramma pretiosum* Riley > *Trichogramma carverae* Oatman and Pinto > *Trichogrammatoidea cryptophlebiae* Nagaraja. We predicted that *T. pretiosum* and possibly *T. carverae* would successfully attack green lacewing eggs but did not expect the specialist parasitoid, *T. cryptophlebiae*, to attack a non-lepidopteran host. No-choice and choice tests under laboratory conditions demonstrated that all three species attacked eggs of *Mallada signata* (Schneider) (Neuroptera: Chrysopidae), a green lacewing species also sold commercially in Australia. However adult parasitoids emerged only from green lacewing eggs parasitised by *T. pretiosum* and *T. carverae*, not from eggs parasitised by *T. cryptophlebiae*. *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), the second host used in choice tests was parasitised only by *T. pretiosum* and *T. carverae*, never by *T. cryptophlebiae*. Our findings suggest that predictions about host specificity of one parasitoid species based on results from a close relative are more likely to be reliable when both species are generalists.

# Risk assessment of the egg-parasitoid *Trissolcus halyomorphae* (Scelionidae) for biological control of the brown marmorated stink bug *Halyomorpha halys* (Pentatomidae) in the USA

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The Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* Yang (Heteroptera: Pentatomidae) was accidentally introduced into the USA from Asia, probably in packing material. It is native to Asia, and was found in Allentown, Pennsylvania in 1998. In 2012 it has spread or been sighted in approximately 37 states including Oregon, California and Florida. In its native range, this pest feeds on a wide variety of plants including fruits, ornamentals, legumes, vegetables, and weedy plants. In the USA the BMSB is causing significant crop losses in orchards and annual row crops. It is expected that the insect's host range will be more extensive as the insect disperses in the USA, and this new invader has the potential to cause significant agricultural losses including temperate and tropical fruits. BMSB survives the winter by invading houses and other enclosed structures. In the spring, BMSB will migrate into crop fields searching for potential host plants where they develop high populations during summer and fall. As part of the risk-assessment, host-specificity tests (choice and no-choice) with the egg-parasitoid *Trissolcus halyomorphae* Yang (Hymenoptera: Scelionidae) from China have been conducted in Florida, Delaware, Mississippi, Oregon and Michigan quarantines during 2011-2012. Approximately 20 species of stink bugs including phytophages and predators in the Pentatomidae, Scutelleridae, Plataspidae, and Cydnidae families have been exposed to the egg-parasitoid in quarantine. The non-target potential risk of the field release of this parasitoid in the USA is assessed.

# Session 3: Recent classical biological control projects against forest pests

## Classical biological control program against the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) in France: objectives and issues

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Considered as one of the most important pest of Castanea species worldwide, the chestnut gall wasp *Dryocosmus kuriphilus* Yatsumatsu (Hymenoptera: Cynipidae) can cause fruit production losses from 60 to 80% (EFSA 2010). Reported for the first time in Europe in 2002 in Italy (Brussino et al. 2002), this pest was detected in France in 2007 and is now widespread in all the main regions of chestnut production (Aquitaine, Corse, Limousin, Midi-Pyrénées, PACA, Rhône-Alpes). Based on previous successful operations in other countries (Cooper and Rieske 2007, Moryia et al. 2003, Quacchia et al. 2008), a classical biological control using the parasitoid *Torymus sinensis* Kamijo (Hymenoptera, Torymidae) has thus been implemented in France since 2011 with three main objectives. The first one is, of course, to durably control the chestnut gall wasp populations, both in commercial orchards and in forests. Another main objective is to use classical biological control as an experimental frame to test hypothesis originating from the field of "invasion biology" (Grevstad, 1999). The propagule pressure - which refers to a combination of the propagule size (i.e. the number of individuals in each introduction event) and the propagule number (i.e. the number of each introduction event) – was thus more precisely investigated as a key factor of the success / failure of small introduced population establishment (Fauvergue et al. 2012, Lockwood et al. 2005, Memmot et al. 2005). The last objective was to document the recruitment of native parasitoids by *D. kuriphilus* and, conversely, the possible impacts of *T. sinensis* on native communities.

During the two first years of this project, 25 releases of *T. sinensis* were made using 2 approaches (one single introduction of 100♀♀ and 50♂♂ versus two yearly introductions of 50♀♀ and 25♂♂ per site). First results indicate that: 1) *T. sinensis* appears to have successfully established in at least 7 of the locations, 2) both *T. sinensis* populations as well as those of native parasitoids, *Bootanomyia dorsalis* (Fabricius) (Hymenoptera: Torymidae), *Eupelmus urozonus* Dalman species group (Hymenoptera: Eupelmidae), *Mesopolobus* spp. (Hymenoptera: Pteromalidae), *Eurytoma* spp. (Hymenoptera: Eurytomidae), etc., display a positive inter-annual growth rate, and 3) no significant impact has yet been observed on *D. kuriphilus* populations.

# Long-term monitoring of the biological control of the European shoot moth *Rhyacionia buoliana* in Central Chile

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*Rhyacionia buoliana* Denis & Schiffermüller (Lepidoptera: Tortricidae) is an exotic pest of the Monterey pine *Pinus radiata* D. Don (Pinaceae) in Chile. The pest was detected in 1985 and soon after a classical biological control effort was initiated, exploring and selecting natural enemies in its European home range. The solitary koinobiont parasitoid *Orgilus obscurator* (Nees) (Hymenoptera: Braconidae) was imported, mass-reared and released across the pine growing area, with great success. Following establishment, a long-term, nation-wide monitoring program was designed and implemented, to track the mortality that *O. obscurator* inflicted on *R. buoliana*. The monitoring was done from 1997 to 2009 at 200-300 plots per year and 8,000-13,000 larvae were dissected each year. Each plot consisted of 16 pines on a 4x4 grid. The trees were examined and apical and lateral bud damage was calculated in the field. Damaged shoots were brought to the laboratory and 30-100 *R. buoliana* larvae per plot were dissected to determine presence of wasp larvae. The efficacy of the biological control agent, expressed as percent of moth larvae parasitized, was calculated for six administrative regions. Differences occurred between years and regions, with a clear pattern of increasing parasitism from South to North. On average, mean parasitism increased from 7% in 1997 to 70% in 2009. The two Southern regions consistently showed higher parasitism than the Northern regions, although the difference decreased with time. The apical damage surpassed the economic threshold before 2001, remained under 10% between 2001 and 2007 and approached the threshold in the last two years of monitoring (2008-2009).

# Classical biological control and *Rhyacionia buoliana*: What we did, what we do and what we need to do?

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*Rhyacionia buoliana* Denis & Schiffermüller (Lepidoptera: Tortricidae) was detected in 1985 in Chile and became one of important pest species in Chile. Population densities increase in a few years until more than 600 larvae per tree were present (including damage of over 10% of apical shoots). After some failed attempts to reduce the advance of the pest with chemical control, the government decided to implement classic biological control as the main method of control. We introduced and released a solitary parasitoid known to be successful in other countries: *Orgilus obscurator* (Ness) (Hymenoptera: Braconidae). However, the hyperparasitoid *Perilampus tristis* Mayr (Hymenoptera: Perilampidae) erupted with force not only in numbers but in behaviour too. We then began to consider the effect of other species, such as the previously detected *Temelucha platensis* Brèthes (Hymenoptera: Ichneumonidae), on the behavior of *O. obscurator* (Lanfranco and Ramos 2010, Ramos and Lanfranco 2010). These two species seem to be competing with *O. obscurator* for the same resource. Recent studies suggest the possibility of apparent competition between *O. obscurator* and *T. platensis*, mediated by the hyperparasitoid *P. tristis*. Data on population levels and parasitism show a moderate increase in moth populations, a slight decrease in the control by *O. obscurator*, and what seems more disturbing, a continued increase in the population levels of *P. tristis* and *T. platensis* in recent years (CPF 2011, 2012).

We don't yet know what the exact effect of these two new parasitoids is on the behaviour of *O. obscurator*, but factors like the emergence of superparasitism and multiparasitism appear to be a good indicator that the biological control is not on the right path. In this presentation we try to clarify what's going on among these three species, the possible future of the pest in Chile using indicators such as apical shoots infestations, levels of parasitism by *O. obscurator* and a new model of the present interactions between *O. obscurator* - *T. platensis* and *P. tristis*.

# *Anaphes tasmaniae*, a parasitoid of *Gonipterus platensis* introduced into Chile

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*Gonipterus platensis* (Marelli) (Coleoptera: Curculionidae) was detected in Chile in 1998, in the Valparaiso region. Since then the Public sector and the Forestry sector are working together to control this pest of *Eucalyptus*. The first parasitoid introduced to Chile was *Anaphes nitens* Girault (Hymenoptera: Mymaridae) from South Africa (1998) and Argentina (2003). The biological control program worked very well during the first years but, when *G. platensis* moved to the south of Chile, regions where the temperature in the winter is below 10ºC, the parasitoid failed to control the pest. For that reason the Servicio Agricola y Ganadero, together with de private sector, began a biological control program based on the search of new parasitoids.

In 2009, about 5,000 egg masses were collected from 20 sites in Tasmania. This material was kept in quarantine until the emergence of *Anaphes tasmaniae* Huber & Prinsloo (Hymenoptera: Mymaridae) adults, which were then used for reproduction, although only a few *A. tasmaniae* emerged. The F1 generation was released at 13 sites, with no recoveries from the field. In 2011, we organized a new collection of parasitoids in Tasmania. About 11,000 egg masses were collected at 17 sites and *A. tasmaniae* was again the only parasitoid to emerge. All the eggs were maintained in quarantine conditions until the emergence of adults. In the *A. tasmaniae* rearing process we used *Eucalyptus globulus* Labillardière (Myrtaceae) bouquets and *G. platensis* adults, which produced fresh egg masses for parasitism. The main problem encountered was the rearing of *G. platensis*, and we did not obtain the required numbers of eggs masses to reproduce the parasitoid in large numbers. To solve this problem the parasitoid was reared at three laboratories (CPF, S.A. CONAF, Mininco) and we finally released the parasitoid at 13 sites. One of these releases was carried out on Rapa Nui Island, but the parasitoid has not yet been recovered. At the other sites, establishment will be assessed next year.

# Quarantine and rearing of *Cleruchoides noackae*, a parasitoid of *Thaumastocoris peregrinus*

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*Cleruchoides noackae* Lin & Huber (Hymenoptera: Mymaridae) is a parasitoid of *Thaumastocoris peregrinus* Carpintero & Dellapé (Hemiptera: Thaumastocoridae), a pest of *Eucalyptus*. Both insects originate from Australia. When *T. peregrinus* was detected in Chile (Region Metropolitana) in 2009, the Servicio Agricola y Ganadero, together with the forestry sector, took the decision to implement a biological control programme against this pest. The same year the pest was detected, contact with the Australian specialist, Dr. Sara Mansfield (University of Sidney) was made to collect the parasitoid *C. noackae* in Australia. In May 2010, eggs of *T. peregrinus* were collected at sites where the presence of *C. noackae* had been reported. The egg masses were kept in quarantine in the Entomology Laboratory of SAG in Santiago of Chile. In the laboratory, we kept a colony of *T. peregrinus* adults to produce egg masses and we did several trials to maintain the egg masses in cold conditions. In total, we were able to produce more than 10,000 egg masses, which were exposed to parasitoid adults. The life span of the parasitoid was about 17-20 days. The release was made at three sites in the Region Metropolitana, consisting in 757 adults and about 18,000 parasitized egg masses. The parasitoid was able to establish at the three sites where it was released. In 2012, *C. noackae* was detected at one site of Valparaiso Region, far away from the release sites.

# Session 6: Mass production of natural enemies: Challenges and Opportunities

## Infectivity of *Aphidius ervi* on different aphid host: Does source matter?

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Parasitoids are frequently used in biological control because of the commonly held belief that they are relatively host specific. As they spend a significant part of their life cycle within their hosts, they are therefore closely related with them, such as that feeding habits of their host can promote specialization via host-race formation (sequential radiation). We examine the case of *Aphidius ervi* Haliday (Hymenoptera: Braconidae) in Chile and the great number of aphid species that it supposedly exploits in different plant hosts and habitats. Infectivity (search and acceptance of the host) and olfactory responses were studied through no-choice and olfactometric experiments, respectively. The parasitoids were reared on four different hosts: *Sitobion avenae* (Fabricius), *Rhopalosiphum padi* (L.), and two clones of *Acyrthosiphon pisum* (Harris) (Ap1 and Ap2) (Hemiptera: Aphididae). The parasitoids of each host origin were tested on their natal host and compared to the other three alternative hosts (non-natal hosts). We found that the parasitoids from *A. pisum* Ap2 and *S. avenae* exhibit greater infectivity on their natal host compared to the alternative hosts (frequency of sting and attack behaviours and time to first sting and attack), suggesting that these are more specialized. The implications of these results for the use of alternative hosts as a refuge in the field and the production of natural enemies for release in biological control are discussed.

# Morphometric characterization of host and habitat associated Aphidius ervi populations

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Parasitoids are frequently used in biological control because of the commonly held belief that they are relatively host specific. As they spend a significant part of their life cycle within their hosts, they are therefore closely related with them, such as that feeding habits of their host can promote specialization via host-race formation (sequential radiation). The specialized host races from different hosts can vary morphologically, behaviorally, and genetically. However, this variation is sometimes inconspicuous, requiring more powerful tools such as geometric morphometrics. We examine the case of *Aphidius ervi* Haliday (Hymenoptera: Braconidae) in Chile and the great number of aphid species that it supposedly exploits in different plant hosts and habitats. The parasitoids analyzed were from different aphid hosts, *Acyrthosiphon pisum* (Harris), *Sitobion avenae* (Fabricius), *Rhopalosiphum padi* (L.), *Schizaphis graminum* (Rondani), and *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae), collected on alfalfa, wheat, lentil, pea, oat and clover between the Maule and Los Ríos regions of central south Chile. Variation of parasitoid wing characters were measured to analyze the variation in shape, using 13 homologous landmarks scored on each individual. At the same time, other relevant size parameters were recorded such as hind-tibia length and average distance to the centroid of wings. Wing shape analyses revealed differences between parasitoids reared from different species of aphids. Wing size of the parasitoids varied according to the size of their aphid host: in this case parasitoids from *A. pisum* were bigger than parasitoids from cereal aphids; an exception being *A. ervi* coming from *A. pisum* on lentils, where parasitiods where significantly smaller that all other biotypes. Results of behavioral and population genetic studies on *A. ervi* are discussed in terms of these results and the implications for identifying biotypes for the mass rearing of specialized host-associated biotypes.

# Development of an artificial diet for the leaf beetle larvae to facilitate mass rearing of larval and pupal parasitoid wasps

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The leaf beetle *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) is one of the most serious pests of coconut palm *Cocos nucifera* L. (Arecaceae) and as a consequence has an effect on the coconut industry, which is an important source of income for farmers in Southeast Asia. Biological control using natural enemies has been attempted to protect the coconut palm from the damage caused by this beetle. Although several natural enemies of *B. longissima* have been reported, two endoparasitoid wasps have potential as biological control agents: the larval parasitoid *Asecodes hispinarum* Bouček and the pupal parasitoid *Tetrastichus brontispae* Ferrière (Hymenoptera: Eulophidae). For secure provision of these parasitoids in biological control programs, a simple method for rearing of *B. longissima* as hosts is crucial. In this study, we developed an artificial diet for *B. longissima* larvae and produced hosts suitable for effectively and continuously rearing *A. hispinarum* and *T. brontispae*. Ingredients in the artificial diet consisted mainly of coconut leaf powder, sucrose, cellulose, minerals and vitamins. When the quantity of coconut leaf powder was increased, the larval developmental period decreased. When L-cysteine was added but cellulose was excluded, the percent adult emergence was 71%. Our artificial diet let to *B. longissima* completing growth from hatching to adult emergence. We next examined the suitability of beetle larvae fed on the artificial diet as hosts for *A. hispinarum* and *T. brontispae*. The percent of wasps that emerged from hosts that were fed the artificial diet was comparable with that from hosts fed fresh coconut leaves, and both parasitoid species advanced to produce wasps of the next generation. Our results suggest that our artificial diet is suitable for rearing *B. longissima* larvae and will facilitate mass rearing of *A. hispinarum* and *T. brontispae*.

# Session 7: Strategies to Increase the Adoption of Biological Control in Greenhouses

## Alternating temperatures regimes affect development rates of *Phytoseiulus persimilis* Athias-Henriot and its target pest *Tetranychus urticae* Koch

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Due to increasing energy costs glasshouse growers are forced to switch to energy saving strategies. In the temperature integration approach, considerable daily temperature variations (DIF= difference between daytime and nighttime temperature) are allowed, which not only have an important influence on plant growth but also on the development rate of arthropods in the crop. In this study we examined the development of *Phytoseiulus persimilis* Athias-Henriot (Mesostigmata: Phytoseiidae) and its target pest *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) at constant and alternating temperatures and at 65 ±5°C RH and a 16L:8D photoperiod. Different temperature regimes were tested at 4 DIF temperature combinations, namely a constant temperature regime and three alternating regimes: DIF+5, DIF+10, and DIF+15 with a temperature amplitude of 5°C, 10°C and 15°C, respectively. Both linear and nonlinear (Briere-2 and Lactin-2) models of development rate versus temperature were used to estimate thermal thresholds and budgets for both mite species. Substantial differences in development rates were observed at alternating temperatures outside of the linear part of the development rate-temperature curve. At a relatively low alternating temperature with a DIF of 15°C (20°C/5°C), development rates were found to increase by 30% for both species as compared to the corresponding mean constant temperature (15°C). At a high alternating temperature with a mean of 30°C (35°C/20°C), the developmental rate of *T. urticae* decreased by 19% as compared to the corresponding mean constant temperature, whereas a 50% lower development rate was observed for the pest's key predator. The differential response of prey and predator to alternating temperature regimes may affect the efficacy of biological control programs.

# Evaluation of different food sources to improve the larval ectoparasitoid *Dineulophus phtorimaeae* de Santis (Hymenoptera: Eulophidae) fitness as a potential candidate for conservation biological control against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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In biological control, parasitoid host-feeding behavior is an important source of host mortality that can substantially reduce a pest population. Through this mechanism hymenopteran parasitoids get a hold on energy required for both maintenance and egg maturation. Besides, many species also require non-host energy sources to improve their fitness. *Dineulophus phtorimaeae* de Santis (Hymenoptera: Eulophidae), a native idiobiont and non-concurrent host feeding parasitoid of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), has been targeted as a potential biological control agent of this pest using augmentative and/or conservation tactics in Argentinean tomato crops. We tested the provision of four food resources to investigate a fitness improvement in *D. phtorimaeae* adult females with the aim of providing useful insights for its mass rearing or habitat design manipulation guidelines. The food sources (treatments) evaluated were honey alone, or combined with soaked raisins, pollen, and a *T. absoluta* larval homogenate. Each type of food was offered to 48 h-old copulated adult wasp females (n=10), until death. Females were provided 8 third instar larvae of *T. absoluta* installed on fresh tomato leaflets every 48 h. Data recorded were: 1) number of alive host larvae (not host-fed or parasitized), 2) number of paralyzed host larvae (host-fed), 3) number of parasitized host larvae, 4) number of days that females lived, and 5) total number of oocytes produced (number of eggs laid plus numbers of oocytes remaining upon dissection of dead wasps). Variables estimated were: mean number of parasitized, host-fed and alive host larvae, wasp longevity and fertility. We found no significant differences among treatments for each variable, except for a higher number of host fed *T. absoluta* larvae by *D. phtorimaeae* fed only with honey (F=4.53, df=34, $p<0.05$). This result indicates that providing other non-host or extra host sources did not improve the attack of *D. phtorimaeae* to its host and inversely, the provision of food probably satiated the wasps, thus preventing higher levels of host feeding and parasitizing.

The ecological importance of feeding on non-host food has been sometimes underestimated. Therefore, efforts to manipulate the availability of alternative food sources must be made in order to improve biological control levels in the field.

# Telematic tools as driving forces to adoption of sustainable practices in greenhouses

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Integrated Pest Management (IPM) is considered as a success story within controlled environment agriculture systems. Nevertheless, biological and integrated control is still used in only 5% of the total world greenhouse area (in 2007). Surveys highlight that potential users of alternative strategies have a strong aversion to risk and difficulties to change their working practices. As a consequence, greenhouse systems still need huge pesticide inputs despite the possibilities it offers in terms of biological, climatic and physical control of the main pests (e.g. thrips, mites, whiteflies, etc.).

In order to help promotion and implementation of such alternative practices we suggest a one hand package of methodologies and telematic tools. Its aim is to help growers to convert their practices and use the possible “bio-control tools” available without depriving them of their own final decision.  This set of tools and methodologies is organized around a specific warehouse database. This database is built according to the “Online Analytical Processing” concept in order to be able to report specific needs of growers or technical advisors.

To support conversion or use of IPM by growers our proposition must be able to support several key steps: 1) acquisition of easy, fast and reliable data, e.g. global quick sampling, video pest detection; 2) aquisition of knowledge expertise and experiences, i.e. a hands-on module for practical pest and disease identification; 3) decision support process, e.g. mapping of epidemics, predictive black box model of dynamics of epidemics, decision rules module.

To design, prototype and validate this global telematic tools accompanying growers in changing practices we are developing global collaborative networks between researchers and growers. Designing this tool in a real context of production with a principle of co-construction with growers must ensure the acceptance and implementation of this tool, resulting in the increasing use of innovative crop protection strategies, especially biological control.

# Can food supplements improve polyphagous predator establishment in vegetable crops?

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*Orius laevigatus* (Fieber), *O. majusculus* (Reuter) (Hemiptera: Anthocoridae), *Macrolophus pygmaeus* Rambur and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) are some of the most common predators used in the biological control of whiteflies, thrips, lepidopterans and other vegetable pests. They spontaneously colonize horticultural crops in temperate areas as well as being mass reared and released for pest control in greenhouses. They are omnivores and feed on different trophic levels, such as different plant products (e.g. pollen, nectaries, etc.) and animal prey. Usually, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs are used as factitious prey to improve predator establishment on crops when pests are scarce, but their use is expensive. In this study, we evaluated the effect of three alternative supplemental foods: dry *Artemia* sp. (Anostraca: Artemiidae) cysts, commercial multifloral bee pollen and the mite *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae). We studied the effect of each food on the fecundity of both *Orius* spp., the fertility of *M. pygmaeus* and *N. tenuis*, the number of chorionated oocytes per female and total longevity of these four predators. Our results show that the use of dry cysts of *Artemia* sp. as factitious prey had similar effects than eggs of *E. kuehniella* on the reproduction of *O. laevigatus*, *M. pygmaeus* and *N. tenuis* and on the number of chorionated oocytes per female of all four predators. On the contrary, the addition of pollen or *T. putrescentiae* did not improve reproductive fitness of any females of these predators. However, dry commercial multifloral bee pollen improved the longevity of the females of these four predators as much as eggs of *E. kuehniella*. In summary, these foods may improve predator fitness and consequently their densities will increase in the crop, enhancing pest control. Dry cysts and dry pollen need little or no handling to be used, are very easy to disperse in the crop, and maintain their quality for a reasonable time.

# *Orius* banker plants, worth the investment?

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In greenhouse ornamental crops, western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), is one of the most economically important and challenging pests to control causing aesthetic feeding damage to plants in addition to vectoring tospoviruses. Insecticide resistance, worker health and safety considerations, and a lack of effective new chemistries have provided incentive to increase the use of biological control agents to control WFT. *Orius insidiosus* Say (Hemiptera: Anthocoridae), is a generalist predator and has been documented as feeding upon a variety of arthropod pests in addition to pollen and plants. Successful control of WFT using augmentative releases of *Orius* has been achieved in greenhouse vegetable crops, such as sweet peppers. The use of *Orius* to control WFT in greenhouse ornamental crops, such as chrysanthemums, has been limited to date as the predatory bug can be slow to establish and exert control – a critical factor with short-term crops. Relatively poor control is achieved in ornamentals as a result of: 1) a lack of prey, as WFT numbers are frequently low in the vegetative stages of plant development; 2) a lack of additional food sources for *Orius*, as ornamentals typically are not flowering in the production area of greenhouses so pollen is not readily available; and 3) the removal of *Orius* eggs from the greenhouse when the crop is shipped, preventing their populations from increasing in the production area. Repeated releases of *Orius* purchased from commercial insectaries by growers are thus necessary, but this approach is not economically viable. Establishing a banker plant system for *Orius* could allow the predatory bug to establish and increase its population in greenhouse ornamental crops. This research project assessed the potential of various banker plant species and evaluated the influence of supplemental food in laboratory and cage trials. In addition, two years of data were collected in commercial greenhouse trials. This poster discusses the results and provides recommendations to make the investment in banker plants for *Orius* pay dividends.

# Preliminary studies of positive and negative effects of brown lacewings on the control of foxglove aphids by parasitoids in sweet pepper

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The use of natural enemy complexes in agricultural systems has been a controversial subject in the management and biological control of pests. Understanding how interactions among natural enemies affect herbivore populations is crucial for biological pest control. Natural enemies can act by different positive and negative mechanism; examples of these are predator facilitation and intraguild predation (IGP). Many aphids drop off the plant as a defensive tactic against predators and parasitoids. But, if aphids drop off the plant after being parasitized this may also facilitate IGP, and the interaction could be antagonistic. We investigated two mechanisms that contradict each other, dropping behaviour (+) and IGP (-), between two natural enemies used to control *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae) on sweet pepper in greenhouses, *Aphidius ervi* Haliday (Hymenoptera: Braconidae) and *Micromus variegatus* (Fabricius) (Neuroptera: Hemerobiidae). Two experiments were conducted, one to see the effects of both natural enemies on the dropping behaviour of *A. solani*, and another to evaluate the preference of the lacewing for unparasitized versus young-parasitized aphids and mummies (IGP). The dropping behaviour experiment showed that in the presence of the parasitoid the proportion of dropping aphids was higher. This can be explained by the spatial niche partitioning, the foraging rates, and the time required to find, manipulate and consume the prey. The IGP experiment shows that adult lacewings had a small preference for young-parasitized aphids over unparasitized aphids, but they preferred unparasitized aphids more than mummies. So, it could be that the IGP was not strong enough to disrupt the suppression of aphids, and both species act additively. The challenge now, based on these studies, is to study the combined effects of *A. ervi* and *M. variegatus* on foxglove aphids in greenhouse conditions to observe the interaction results on the control of *A. solani*. The results of this study show the importance of understanding the various types of interactions than can occur between predators and parasitoids when assessing their value for biological control, especially in greenhouse systems where biodiversity can be managed.

# Has tiger-fly a role in biological control of protected crops?

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The tiger-fly, *Coenosia attenuata* Stein (Diptera: Muscidae), distributed worldwide, is a promising predator of important greenhouse pests. Larvae prey on soil-dwelling organisms, and adults attack insects in flight, killing more individuals than they actually consume. Open rearing units could be used in inoculation biological control and for enhancing conservation biological control. The “three-step” rearing methodology described in the literature was adapted/modified in order to optimize the rearing methodology in terms of quantity and quality of the individuals produced, costs and time consumed. Adults were reared on sciarid (fungus gnats) and drosophilid adults, and larvae on sciarid larvae. A higher number of *C. attenuata* emergences occurred on a highly humidified rearing substrate, infested with abundant sciarid larvae, composed of soil rich in organic matter, mixed with oat flakes inoculated with the fungus *Pleurotus ostreatus*(Pleurotaceae), and with coconut fiber. Rearing cages with 10 parent couples gave good results, and cannibalism was avoided when adult prey was abundant. In laboratory conditions, earthworm mucus and sciarid larvae significantly induced the *C. attenuata* females to extend their ovipositors more frequently and to lay more eggs. The predation capacity and behaviour of *C. attenuata* was studied in the laboratory in relation to several pests, parasitoids and predators, and all taxa tested were preyed upon: *Diglyphus isaea*, *Trialeurodes vaporariorum*, *Liriomyza huidobrensis*, *Drosophila melanogaster*, *Eretmocerus mundus*, *Nesidiocoris tenuis*, *Orius laevigatus*, *Dacnusa sibirica*, *Pseudococcus viburni*, Thripidae and Psycodidae. Small adults of *Tuta absoluta* could also be prey. There was some evidence of female preference for whiteflies and leafminers in relation to *D. isaea*, and predation behaviour for whiteflies differed when leafminers or drosophilids were present. These studies were supported by Portuguese national funds provided by the FCT (PTDC/AGR-AAM/099723/2008).

# Session 8: Augmentative and conservation biological control techniques in forestry

## Formosan subterranean termites and their interaction with entomopathogenic fungi

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Formosan Subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), is the most important pest that infests economically important timber crops and structures such as houses, other buildings, boats, utility poles and underground telephone cables. Because of their cryptic habitat, it is difficult to manage the termites. Naturally-occurring entomopathogenic fungi are thought to act as a promising alternate for management of *C. formosanus* because they are living in soil that is also a favorable habitat for fungal growth. Despite more than 40 years of intensive successful laboratory investigations of entomopathogenic fungi, colonies of *C. formosanus* are uniquely non-susceptible to fungal infection. Reasons for this lack of success include both behavioral and physiological mechanisms that termites have evolved to resist infection against disease causing agents. Behavioral mechanisms include the use of allogrooming to remove fungal conidia from the surface of the cuticle of nest mates, pathogen alarm behavior which warns nest-mates about the presence of lethal fungi causing them to leave the area, and antifungal chemical defenses. Until now, little work has focused on the physiological adaptations which is mainly under the control of humoral immune responses among *C. formosanus* workers in response to microbes to resist the infection among the nest mates. The humoral immune response of *C. formosanus* mainly includes: 1) identification of pathogen-associated molecular patterns (PAMPs) on pathogens by pattern recognition receptors (PRRs), 2) signal modulating and transducting molecules to activate the signaling pathways, such as the Toll and Imd pathways, and 3) production of immune effectors such as antimicrobial peptides (AMPs). In conclusion, understanding the host pathogen interaction at every level is important in order to devise an Integrated Pest Management strategy.

# Biological control agents of cypress aphid present in Chile

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The cypress aphid *Cinara cupressi* Buckton (Hemiptera: Aphididae) is considered one of the hundred most invasive pests in the world. In Chile, it was detected in 2003 in Pica, Región de Tarapacá, and today it is currently distributed throughout the national territory, attacking native and exotic species of Cupressaceae. To mitigate damage to host trees and control this pest the Servicio Agrícola y Ganadero distributed the parasitoid *Pauesia juniperorum* Starý (Hymenoptera: Braconidae), found in 2004 in the Regiones de Valparaíso and Metropolitana, to 21 different places where the parasitoid was absent, from the Region de Atacama to the Region de Los Lagos. From 2005–2007, a total of 990 specimens of the parasitoid were released. However, so far, the parasitoid has not achieved the control expected through 2012 and there are only nine places where the parasitoid has been found. As result of surveys conducted to document the establishment and distribution of parasitoids and other natural enemies in Chile we have found *Syrphus* sp. (Diptera: Syrphidae), *Adalia bipunctata* (Linnaeus), *A. angulifera* Mulsant (Coleoptera: Coccinellidae) and Hemerobiidae (Neuroptera) larvae. All are generalist predators showing some degree of control but they are not satisfactory in decreasing the aphid populations. Entomopathogenic fungi such as *Verticillium lecanii* (Zimm.) Zare & W. Gams (Plectosphaerellaceae) and the recently described *Neozygites osornensis* Montalva & Barta (Neozygitaceae) were also found in the surveys. The latter are promising agents and are being studied to complement the use of parasitoids and other natural enemies and effectively reduce pest *C. cupressi* populations.

# Will climate change affect biological control in forests and other natural habitats?

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Natural enemies play an essential role in regulating forest insect populations and preventing pest outbreaks. Climate change may affect their action, either through a direct effect on the natural enemy itself or through disturbance of pest-natural enemy interactions. Two major issues in studying such effects are, firstly, the fact that studies tend to focus on single species and, therefore, results are difficult to extrapolate to other species. Secondly, climate change is a slow process and, ideally, observations should to be done over a long period of time, which is rarely possible. In the EU-funded project BACCARA, we used various approaches to assess the effect of climate change on parasitoids of forest pests and on their regulatory effect on forest pest populations. In particular, we used the concept of functional groups and elevation gradients as analogues of global warming. We carried out meta-analyses to derive common responses of parasitoids and their herbivorous hosts to elevation. While the response of herbivorous insects to elevation is idiosynchratic, parasitism rates and parasitoid species richness significantly decrease with increasing elevation. The decrease is greater for ectoparasitoids and parasitoids of ectophagous insects than for endoparasitoids and parasitoids of endophagous hosts, possibly because these latter are better protected from adverse and extreme climatic conditions occurring at higher elevations. Although our results suggest an increase of parasitism with increasing temperature, other factors regulating herbivorous insects have to be considered before concluding that climate warming will lead to a decrease in pest density.

# Session 9: The effect of climate change on biological control

## Environmental conditions affect intraguild predation: implications for pest biological control in avocado orchards

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The change in environmental conditions predicted by climate change models may profoundly affect agricultural communities under biological control management. The capacity to predict the effect of global warning in agricultural communities depends on the identification of interactions between species that are vulnerable to environmental conditions and key to the maintenance and the performance of the community. We investigated the effect of warming in a community present in avocado orchards in South-eastern Spain. In coastal areas, with relatively mild environmental conditions, this community is mainly composed of the herbivore pest *Oligonychus perseae* Tuttle, Baker and Abbatiello (Tromibidiformes: Tetranychidae) and two mites that are natural enemies of the pest: *Neoseiulus californicus* (McGregor) and *Euseius stipulatus* (Athias-Henriot) (Mesostigmata: Phytoseiidae). In this work we determined experimentally the role played by environmental conditions on the strength and direction of predatory interactions among the components of the community. Our results suggest that increasing temperatures and drought will probably affect the performance of both predators as natural enemies of the pest.

# Environmental warming influences the relative competitive ability between two natural enemies sharing a common prey

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It is known that environmental warming can deeply affect the strength of interactions such as competition and predation. In this work, we examine whether variation in predator-predator interaction strength (i.e. competition and/or predation) caused by environmental conditions could explain the observed distribution and abundance of two sister species. These two species are the phytoseiids *Euseius stipulatus* (Athias-Henriot) and *E. scutalis* (Athias-Henriot) (Mesostigmata: Phytoseiidae). Both species colonize avocado orchards and prey on the persea mite, *Oligonychus persea* Tuttle, Baker and Abbatiello (Tromibidiformes: Tetranychidae), and feed on pollen when the prey is absent. However, *E. stipulatus* colonizes avocado orchards of areas with mild environmental conditions, whereas *E. scutalis* is found in orchards of areas with more extreme climate. We carried out laboratory experiments at the individual and population levels under different environmental conditions. Results showed that the oviposition rate of *E. stipulatus* is affected by temperature and relative humidity, whereas that of *E. scutalis* is affected only by temperature, and at much lesser extent. At the population level, we first determined whether superiority at exploitative competition was climate-dependent. Results suggested that when the two species co-occur under hot and dry climate scenarios, *E. scutalis* should always exclude *E. stipulatus*. Community dynamic experiments with the two species together at hot and dry environmental conditions confirmed this hypothesis, which agrees with the pattern observed in the field. At milder environmental conditions, both species could exploit the resource with similar efficiency. However, the tendency in the community dynamics experiments was towards *E. scutalis* being excluded by *E. stipulatus*, a result that also agrees with observations in the field, but that cannot be explained by competitive abilities only.

# Potential future distribution of the multicolored Asian lady beetle, *Harmonia axyridis* (Pallas, 1972), in Chile under a global warming scenario

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*Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is a generalist predator, native to Asia, that has been introduced to several parts of the world for the biological control of aphids, acquiring characteristics of an invasive species. In central Chile, wild populations were not found until 2008, and now it is present over a ~1000 km. range from the Atacama to Araucanía regions. It is believed that this invasion is still in process, so it would invade new locations, which also depends on the physiological tolerance of *H. axyridis* to a thermal increase, due to global warming. Laboratory experiments have shown that the fitness of *H. axyridis* adults (metabolic performance and survival) is lower at temperatures above 28°C, and eggs do not hatch at 33°C. Because climate warming may interact with the invasion probability of this species, in this paper, we modeled the potential distribution of *H. axyridis* in Chile, based on its current distribution and 19 present and future (year 2080) bioclimatic variables, considering climate warming (Worldclim.org). We expected that the potential distribution modeled with future bioclimatic variables (i.e future model) would be limited, in terms of range and/or probability values, compared with the model constructed using present climatic variables. The models were run with the MAXENT 3.3., software based on the maximum-entropy approach for species modeling. Present and future models show a potential distribution predominantly on central and extreme south Chile. Future model results in an increase of potential distribution range compared to present, especially south and east, and expansion is greater in central (~300 km. to the south) than in the extreme south Chile. However, probability values decrease significantly from present to future (maximum probability of occurrence points used for modeling in present are ~72%, and in future ~38%). Average temperature of the warmest month of every year, for the central Chile potential future distribution is 29°C, which is on the decreasing curve zone of the fitness curve, so potential distribution of *H. axyridis* may be constrained even more in the future. These results mostly support our hypothesis and suggest that global warming will interact antagonistically with the invasion of this species in Chile. Funding: FONDECYT 1100159

# Influence of temperature on the life cycle of *Harmonia axyridis* Pallas

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*Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), due to its present high predation potential, has been used in biological control programs for pests. Temperature variation plays an important role in the development of this predator and, consequently, their predation ability. This study eamined the life cycle development (egg to adult) of *H. axyridis* at three different temperatures. Individual eggs (30) of *H. axyridis* were placed in glass tubes, 8.0 cm x 2.5 cm, at each constant temperature of 18, 24 and 30ºC ±1ºC, 14 h photoperiod and 70% ±5% relative humidity. The larvae were reared in that container and fed daily with *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae). After adult emergence, couples were kept in transparent plastic 250 ml cups (7.0 cm x 10.0 cm), sealed with fabric "voile", and provided with the same type of food given to larvae. Through daily observations we evaluated the effect of temperature on each phase of development of the ladybird, and used multivariate analysis (cluster analysis and two entries) to analauze the data. It was found that temperatures of 24 and 30ºC were favorable to the development of *H. axyridis*, while the temperature of 18ºC acted adversely on all phases of development.

# Session 10: Use of Volatiles to Monitor or Manage Natural Enemies

## Natural enemy responses to the sex pheromone of *Pseudococcus viburni* in laboratory and field conditions

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Mealybugs (Hemiptera: Pseudococcidae) are important pests in fruit orchards and vineyards worldwide. Visual mealybug monitoring is difficult because of their cryptic biology, as well as for their biological control agents. Sex pheromones from several mealybug species have been identified and synthetized, and have been used for a decade to monitor mealybugs. Parasitoid response to mealybug sex pheromones have been reported for some mealybug species. *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae) is the most abundant mealybug species in Chilean vineyards. Unfortunately, there is no data about the response of *P. viburni* parasitoids or other natural enemies to its sex pheromone. The aim of this work was to determine if the *P. viburni* specific parasitoid *Acerophagus flavidulus* (Brèthes) (Hymenoptera: Encyrtidae) and the mealybug predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) respond to *P. viburni* pheromone in laboratory and field conditions. Laboratory experiments were conducted using adult parasitoid females as well as adult and larvae of the predator. Insects were placed in a Y tube olfactometer, with a septum containing 25 µg of the synthetic pheromone and a blank. The choice made by the individuals and the time of response were recorded with the program JWatcher 0.9. In the field, three pheromone traps loaded with *P. viburni* sex pheromone were placed in four vineyards during the 2008-2009 season. To test the specificity of the response, pheromone traps loaded with the sex pheromone of *Planococcus ficus* (Signoret), *P. kraunhiae* (Kuwana) and *P. minor* (Maskell) (Hemiptera: Pseudococcidae) (species not known to be present in Chile) were also placed in each vineyard. Pheromone traps were changed every two weeks and analyzed under a microscope. Male mealybugs and parasitoids were counted and recorded. The results showed that in laboratory conditions *A. flavidulus* was attracted to *P. viburni* sex pheromone, but not the predator. In field conditions, *Acerophagus* was the main genus of parasitoid found in traps loaded with *P. viburni* pheromone, but no parasitoids were recorded in the traps with pheromone of the other *Planococcus* species. In one of the vineyards, significant correlations were found between mealybug males and parasitoids found in traps ($R^2 = 0.51$; $p < 0.05$). Also no *C. montrouzieri* were found in the traps. These results show that mealybug sex pheromone acts as a kairomone in the case of parasitoids, but not for predators, who would be responding to non-species-specific cues. Therefore these pheromones have the potential of being use as a tool to monitor and manage specific parasitoids of this important pest.

Funding: Fondecyt 1080464, Fondef D10I1208, CONICYT Doctoral Fellowship 21110864, Conicyt Grant “Tesis de Postgrado en la Industria 7812110011”.

# Session 11: Integrating Conservation Biological Control and Wildlife Conservation

## Mite diversity in shaded coffee and under full sun

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Agroecosystems with a diversity of plants can result in increased opportunities for environmental conditions favorable to natural enemies and therefore conservation and increased biological control, since the natural vegetation near the crops provide suitable habitats for the preservation of natural enemies. This study aimed to obtain knowledge of mite fauna on shaded coffee plants, *Coffea arabica* L. (Rubiaceae), naturally found in the interior of forest fragments, and coffee grown under full sun, at least 100 m from forest fragments. The evaluations consisted of one collection of leaves per year for two consecutive years, made after the dry period of each year. In the forest fragment, leaves were collected from coffee trees naturally found within the fragment, for a total of 45 leaves, 15 in each of the three sample units. From coffee grown under full sun, 45 leaves of 'Catuaí' coffee, 15 in each of the three selected sampling units were also collected. The leaves were placed in plastic bags and were washed in the laboratory to remove mites, which were preserved in 70% alcohol in plastic vials. Later, the mites were mounted on slides with Hoyer medium, for identification, initially to the family level. The predatory mite family Phytoseiidae (Mesostigmata) showed the highest average number of mites per coffee leaf, being 1.6 in the shade and 0.5 under full sun, followed by the Tydeidae (Trombidiformes) mites that have varied feeding habits (0.88 in the shade and 0.78 under full sun) and thephytophagous Tenuipalpidae (Trombidiformes) mites (0.88 in the shade and 0.36 under full sun). In addition to the higher average number of mites per leaf, the Phytoseiidae, under shade, was also dominant, very abundant, very frequent and constant. The order Oribatida, cosmopolitan and with diverse habits, mainly including plant material and fungi decomposer species, phytophagous, entomophagous, nematophagous, mycophagous, lichenophagous, pollenophagous and bacteriophagous species, showed the fewest numbers under full sun (0.05 mites/leaf) compared to numbers in shaded coffee (1.6 mites/leaf). Therefore, the preservation of forest fragments near coffee plantations is suitable for biological control by conservation.

# Native carabid beetles and salamanders as natural enemies of the invasive earthworm *Amynthas agrestis*

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The introduction of non-indigenous species is amongst the most serious dangers for species diversity around the world and this raises tough questions for the protection of wildlife and natural reserves. Should and can we protect natural native ecosystems from invading alien species? Crucial for such a decision is a profound knowledge about interactions between invasive species and native species communities. In this study we investigate the interactions between native predators, especially carabid beetles and salamanders, and an invasive earthworm in the Great Smoky Mountain National Park. Asian earthworms of the genus *Amynthas* (Haplotaxida: Megascolecidae) have been introduced unintentionally to the National Park, which is well known for its high species diversity. Besides analyzing changes in the soil-dwelling species community, the study investigated the trophic interactions between the most abundant predators and the invading earthworms. Many of the most active predators found in the study area are known as generalists and it seemed likely that they will feed on the invading earthworms and may even act as natural control agents. Using specially designed molecular markers, we were able to identify those carabid beetles and salamanders that feed on *Amynthas agrestis* (Goto and Hatai). We also investigated changes in the overall diet of these generalist predators to detect crucial changes in the food web. Special emphasis was given to the development of non-invasive approaches, which enable further investigations into the potential of native predators in regulating the invasive earthworms without killing the predators. This is especially important for studies in protected areas, which usually contain rare or protected species and require minimizing negative impacts by research.

# Session 13: Functional implications of non-prey feeding in biological control

## Interactions of *Ricoseius loxocheles* (Mesostigmata: Phytoseiidae) and coffee leaf rust

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Coffee crops have economic losses due to pests and diseases. Among them, the phytophagous mites deserve attention because they reduce photosynthetic areas on the leaves. Besides mite damage, some pathogens also attack coffee plants, e.g. the coffee rust fungus, *Hemileia vastatrix* Berk. & Broome. This pathogen causes yield losses around 30% in some varieties of *Coffea Arabica* L. (Rubiaceae). Among the Phytoseiidae, carnivorous and pollen-feeding mites, we surveyed in the field for *Ricoseius loxocheles* (De Loan) (Mesostigmata: Phytoseiidae) on necrotic areas caused by coffee rust during its reproductive phase. We investigated the taxonomy, development, survivorship and reproduction parameters of *R. loxocheles* feeding on coffee rust fungus, its predation capacity on the herbivore mite *Oligonychus ilicis* (McGregor) (Trombidiformes: Tretranychidae), and its reproductive success. Coffee rust supported the survival, development and reproduction of *R. loxocheles*. However, the mite was not able to feed on different stages of *O. ilicis*. Survival and oviposition of *R. loxocheles* was only observed when fed with fungi. Additionally, the fertility of *R. loxocheles* fed on coffee rust was higher than other phytoseiids fed on other fungi or pollen. It is possible that *R. loxocheles* has a role in the control of coffee rust since it feeds on large amount of rust uredospore.

# Session 14: Generalist predators for plant pest control: applications, prospects and drawbacks

## An analysis of population structure of two *Orius* species in Japan for conservation biological control

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Conservation of natural enemies around agricultural fields has recently attracted considerable attention in Japan. Although habitat management is an important in conservation biological control, how wide habitat should be managed has not been clear so far. Molecular ecological approach employing DNA markers will contribute to elucidating this problem.

Minute pirate bug (*Orius*) is one of the most promising predators in controlling small insect pests. Four (major) species of *Orius*, *O. strigicollis* (Poppius), *O. sauteri* (Poppius), *O. minutus* (L.) and *O. nagaii* (Yasunga) are distributed in mainland Japan, and habitat preferences are different among them. To conserve them sustainably, their habitat size should be determined. In this study, we compared population genetic structure of *O. strigicollis* and *O. sauteri* in northern Kanto area in Japan using microsatellite DNA markers. Relationships between pairwise geographic and genetic distances among populations were compared. *Orius strigicollis* showed clear positive correlation between the two distances while *O. sauteri* had no clear genetic structure. The effect of mixing genes among populations becomes strong if dispersal occurs frequently, and consequently genetic differentiation among populations will disappear. According to this theory, dispersal of *O. sauteri* is probably more frequent than that of *O. strigicollis*, and their habitat size might be larger than that of *O. strigicollis*. In contrast, much narrow area should be considered for conservation of *O. strigicollis.*

# Native predators control *Bactericera cockerelli* in potato crops in New Zealand

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The recent arrival of *Bactericera cockerelli* (Sulc) known in New Zealand as tomato-potato psyllid (TPP) and its associated bacterial pathogen, Candidatus Liberibacter solanacearum Jagoueix et al. (Rhizobiaceae) has curtailed the development of an IPM programme for potatoes. The potato industry has been forced to increase the use of calendar applications of insecticides and research on management of, in particular, *Phthorimaea operculella* (Zellier) (Lepidiptera: Gelechiidae), potato tuber moth (PTM), has been redirected to assess existing natural enemies in potatoes and their impact on TPP. Three years of weekly monitoring of untreated potatoes and spring and summer field trials over four years in south Auckland, in the north of New Zealand, show that there are existing biological control agents (BCAs) that give substantial control of all pests in potatoes. The most common and most important BCAs are the two foliage-dwelling native predators, *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae) (brown lacewing) and *Melanostoma fasciatum* (Macquart) (Diptera: Syrphidae) , known as small hover fly, that control aphids, exposed lepidopteran larvae and TPP for up to 8 months of the year. *Micromus tasmaniae* is present all year and becomes common in spring and summer, while *M. fasciatum* appears in crops in early summer and becomes the dominant predator peaking at up to 200 eggs and larvae per plant. However, large flights of TPP adults may occur from January through to April (summer through to autumn) leading to large populations of TPP nymphs infesting plants that together cause major crop losses to unprotected crops. Lower populations of TPP in cooler regions further south provide an opportunity for naturally occurring BCAs to give longer-lasting control of TPP. Laboratory choice and no-choice studies on the most common predator species, that include *Coccinella undecimpunctata* (L.) (Coleoptera: Coccinellidae) and *Nabis kinbergii* Reuter (Hemiptera: Nabidae) indicate that all life-stages of these foliage dwelling predators are capable of consuming all life stages of TPP even in the presence of aphids. However, in field crops *M. fasciatum* may displace *M. tasmanaie* due to intra-guild predation. The potato industry urgently requires the availability of compatible insecticides for maximising the impacts of these important BCAs, plus other validated IPM tools for sustainable production of potatoes, particularly for the longer (warmer) growing regions in New Zealand.

# Does predator functional richness increase the biological control of plant pests?

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Determining when multiple predator species provide better prey suppression is a key step towards developing conservation biological control strategies. While numerous studies have demonstrated that diverse predator assemblages can be more effective at controlling pest population, other studies have shown that it is the presence or absence of a competitively superior species which is critical to pest biological control (i.e. selection effect).

We carried out a manipulative experiment to test the hypothesis that increasing functional richness of ground beetles (Coleoptera: Carabidae) increases prey suppression. Beetles were a priori grouped in three functional groups of different size based on their prey’s size. Predation on an invertebrate prey community was compared between nine created assemblages containing one, two, or three different functional groups of beetles. Preys used were adults and eggs of *Deroceras reticulatum* (Müller) (Agriolimacidae), eggs of *Helix aspersa* (Müller) (Helicidae), larvae of *Tenebrio molitor* L. (Coleoptera: Tenebriondidae) and *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae)

We found no evidence that increasing ground beetle functional richness increases prey suppression. Instead, our results showed that the identity of functional groups was more important in determining the ability of ground beetle communities to prey suppression. Predation efficiency was positively correlated to the presence of large beetles. The presence of *Carabus auratus* (L.) (Coleoptera: Carabidae) was particularly important. Furthermore, multivariate analyses revealed distinct patterns in resource use among functional groups. Small beetles tend to attack small preys, while largest one showed maximum efficiency of predation on all preys. Our results are consistent with other multiple-predator studies and suggest that, for the biological control of plant pests, conservation strategies that directly target key species of ground beetles will be more effective than those targeting these predator biodiversity.

# *Chrysoperla externa* (Neuroptera: Chrysopidae) as a biological control agent of pests in roses grown in an integrated production system

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The rose is the most produced and merchandised ornamental species worldwide. In Brazil, this crop stands out with about 180 millions of stems marketed per year, and Minas Gerais has one of the largest productions of the country. One of the biggest challenges faced when growing flowers and ornamental plants is related to pest control. That is because any injury caused by phytophagous arthropods is unacceptable as it depreciates the final product (flowers and leaves) which will be marketed. The Integrated Production System proposes the preservation of natural resources prioritizing safer methods to farmer's and consumer's health, and at the same time less harmful to the environment. This system involves a set of agricultural practices which optimize the cultivation with the aim of increasing productivity and improving the quality of the produced flowers. The goal of this research was to monitor the fluctuation of the pest population in roses by releasing eggs and larvae of the predator *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) in a growth kept under Integrated Production System. Plants of the variety 'Carolla' were grown in a greenhouse of 19.2 m x 24.0 m spacing of 1.20 m x 0.25 m. The total area was divided in two equal parts and in one of them three rows of *Calopogonium mucunoides* Desv. (*Fabaceae*) to be used as green manure was sown between the rows of the roses. The control of the phytophagous arthropods by the predator was evaluated by weekly samplings during six consecutive months. It was found mites, *Tetranychus urticae* (Koch) (Trombidiformes: Tetranychidae), the aphids *Macrosiphum rosae* L. and *Macrosiphum euphorbiae* Thomas and whiteflies, *Bemisia* sp. (Hemiptera: Aleyrodidae), whose populations were in relatively low density due to control by *C. externa* larvae. The population density of mites and aphids was significantly lower in the area where *C. mucunoides* was planted, showing that the association with the green manure caused a negative effect on these pests. Thus, the Integrated Production System using *C. externa* and *C. mucunoides* can contribute to improve the quality of flowers, without the necessity of chemical products for controlling of pest organisms.

# Selection of candidate plants for the conservation of *Orius* predators

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Vegetable production in the Mediterranaean area is highly intensive and crop cycles are rather short. Conservation biological control is increasingly being used in the area but the periodic destruction of crop and non-crop vegetation hinders the establishment of natural enemies that must re-colonize fields each time. In this scenario, the establishment of ecological infrastructures with selected plant species that improve habitat and resources availability to key natural enemies would ensure their presence close to the crops and enhance biological control of pests. Many of the beneficials most widely used are generalist predators and among them *Orius* spp. (Hemiptera: Anthocoridae) are of special relevance. The aim of the study was to find out the plants that would be more useful to act as a refuge and reproduction site for native *Orius* species, especially early in springtime when populations of beneficials are low. From February to July we monitored twenty-five plots of selected plants, native or being used as ornamentals, from those mentioned in the bibliography as of interest for *Orius* conservation. Our results show that *Orius* spp. were present in almost all plant species sampled, but only in 7 *Orius* were detected at significant level. Overall, the highest population was recorded in basil, *Ocimum basilicum* L. (*Lamiaceae*), but only when the plants were blooming, which occurred late in the sampling period. On the other hand, in sweet alyssum, *Lobularia maritime* (L.) Desv. (*Brassicaceae*), *Orius* sp. adults were already found at the first sampling and nymphs from the beginning of May, indicating that this plant is interesting not only as shelter for adults but also as a reproductive host. Regarding *Orius* species composition, four species were found: *O. laevigatus* (Fieber), *O. majusculus* Reuter, *O. niger* (Wolff) and *O. albidipennis* (Reuter). All four species were present in basil, being *O. laevigatus* the most abundant one. In sweet alyssum, the same species were found except *O. majusculus*, and *O. laevigatus* was again the most present. Other potential natural enemies were also sampled to know the overall potential of these plant species to conserve and enhance the population of a wider range of natural enemies.

# Intraguild predation and cannibalism on eggs of native and exotic coccinellids in alfalfa fields, in central Chile

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Introduction of species is factor for biodiversity loss, as native species may be displaced by the exotic ones. Mechanisms moderating these effects are poorly understood. Theory predicts that coexistence of species in the same guild would be possible if negative intraspecific interactions effects (cannibalism, CAN) are greater than interspecific (intraguild predation, IGP). In Chile, populations of the ladybug *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) have increased since its introduction in 1975 for biological control, whereas abundance of native species *Eriopis connexa* (Germar) (Coleoptera: Coccinellidae) has declined. We evaluated IGP and CAN interactions on eggs of these two coccinellids. Sentinel eggs were placed on cards in four alfalfa fields located in Pirque (20 km South of Santiago, Chile), during spring, summer and autumn. We counted consumed eggs at three hours intervals for 24 hours and estimated predation. Predators observed feeding on eggs were identified or collected for later identification. Prey and predators abundance were evaluated with yellow sticky cards, sweep nets and pit fall traps. All predators observed feeding on eggs exhibited taxa specific diel patterns of predation, and were primarily diurnal. Proportion of predators that fed on eggs was different from their proportion in abundance in alfalfa fields. Seasons did not affect the occurrence of predation on eggs on either species, but day time did, reaching a peak at 4pm by *E. connexa* and *H. variegata* adults. Both coccinellids species eggs showed similar IGP to CAN ratio, but total predation when considering all predators was higher on *E. connexa* eggs. Therefore the native species is more susceptible to predation than the exotic. Funding FONDECYT 1100159.

# Capacity of predation of *Cycloneda sanguinea* (Linnaeus) (Coleoptera: Coccinellidae) fed on *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae)

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*Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) is cosmopolitan, occurring in the Americas and Europe. Some studies have reported the species as one of the main predators of aphids in cotton, sugar cane, citrus, mango, soybeans and sorghum crops. The aim of this study was to evaluate the potential of predation of *C. sanguinea*, having as prey the aphid *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) in sorghum plants. We determined the daily capacity of predation of larvae and adults of the ladybug, i.e., the number of aphids consumed by each development stage of the coccinellid. The densities of prey offered to the predator larvae ranged from 20 nymphs for the first instar to 450 nymphs to the fourth instar. We evaluated the total intake during each instar and larval stage of the ladybugs, while the predatory capacity of adults was conducted during the period of 10 days. Newly hatched larvae were held individually and fed daily with nymphs and adults of *S. graminum*. These insects were kept in climatic chambers at 25° C ± 1.0° C, 12 h photophase and 70 ± 10% relative humidity. The capacity of predation of *C. sanguinea*, in the 1st, 2nd, 3rd and 4th instars averaged 43.5; 84.1; 86.6 and 601.1 aphids, respectively. During the larval stage, the species consumed on average 1115.3 aphids. Adult ladybirds consumed 146.7 aphids daily and over 10 days consumed 1466.9 aphids. *Cycloneda sanguinea* proved to be efficient predator of *S. graminum*, with potential to control this pest.

# Predatory capacity of ground beetles (Coleoptera, Carabidae) captured in agroecosystems in northeast region of São Paulo State, Brazil

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In this study, adult carabids were evaluated to compare the consumption pattern of 4th instar larvae of *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae). The ground beetles were captured in four areas of forest fragment and soybean / corn crops or orange orchard which were located in three municipalities of the northeast region of São Paulo State, Brazil. The prey consumption was calculated based on the dry weight of larvae that was determined by the regression model ($y = -0.001224 + 0.170274x$, $R^2 = 0.91$), which considered the fresh weight (x) and dry weight (y) of 20 4th instar larvae of *A. gemmatalis*. The pattern of prey consumption was estimated for carabid species that caused at least 80% mortality of prey offering a 4th instar larvae every 24 hours, during four consecutive days. Analysis of variance (ANOVA) was used to detect significant differences in the consumption pattern of carabid species, the means being compared by the Tukey test ($P < 0.05$). Among 62 species of carabids, 28 species (45.2%) caused 50 to 100% mortality of prey, consuming 0.0004 to 0.0082 mg dry weight in 24 hours. Several *Selenophorus* spp. (Coleoptera: Carabidae) showed no ability to kill the prey and may represent species that consume seeds of herbaceous plants. Generally, the prey consumption by carabid species showed no significant differences in the four days considered. The results indicate a great capacity of prey consumption by these carabid species which can be availed for the development of biological control programs.

# Diversity of ground beetles (Coleoptera, Carabidae) in forest fragments and soybean/corn crops under two tillage systems

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The objective of this study was to determine the species composition and diversity of carabids in areas of forest fragment and soybean/corn crops. The study was carried out in three areas located in two municipalities in São Paulo State, Brazil, from November 2004 to December 2007. The area, in Jaboticabal municipality, was located at the Paulista State University Campus (21°14'52'' S, 48°16'04'' W). The site comprised 40 ha of cultivated with soybean, *Glycine max* (L.) Merrill (Fabaceae) rotated with corn, *Zea mays* (L.) (Poaceae), in a no-tillage system adjacent to 15 ha of semi-deciduous broadleaf tropical forest fragment. Two areas were located in the Guaíra municipality, about 2 km from each other. One area (20°21'10'' S, 48°14'47'' W) comprised a 90 ha field cultivated with soybean rotated with corn in a no-tillage system, adjacent to 48 ha of semi-deciduous broadleaf tropical forest fragment. A second area (20°19'29'' S, 48°15'08'' W) had 15 ha cultivated with soybean rotated with corn in a conventional tillage system, adjacent to 6 ha of semi-deciduous broadleaf tropical forest fragment. Insects were sampled using pitfall traps arranged along two parallel transects 200 m long, placed across the crops/forests boundary, and extending 100 m into each habitat. The Shannon-Wiener diversity and Morisita similarity indexes were calculated. A total of 2,936 individuals of 65 carabid species were captured. The carabid diversity was higher on the edges than inside the forest fragments or soybean/corn crops while the carabid communities in the no-tillage areas were better structured compared to the conventional tillage. On the other hand, the carabid communities observed in the forest fragments and crop fields typically showed the lowest similarity while the highest similarity levels occurred in communities inhabiting the fragment and edge of the conventional tillage or the crop field and edge in a no-tillage area.

# Determining ground beetle (Coleoptera: Carabidae) consumption of lowbush blueberry insect pests

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Generalist predators provide agroecosystem services if they consume plant pests. The most common ground beetles in lowbush blueberry fields in Nova Scotia, Canada were evaluated as predators of blueberry spanworm, *Itame argillacearia* (Packard) (Lepidoptera: Geometridae), blueberry flea beetle, *Altica sylvia* Malloch (Coleoptera: Chrysomelidae), and blueberry maggot, *Rhagoletis mendax* Curran (Diptera: Tephritidae), common pests in this system. In the laboratory, *Carabus nemoralis* Müller, *Pterostichus mutus* (Say), and *Poecilus lucublandus lucublandus* (Say) (Coleoptera: Carabidae) consumed spanworm, but rates for the first two species were significantly reduced in cups with greater structural complexity (soil + blueberry stems vs. only soil). We observed that *P. lucublandus lucublandus* can climb plants, and spanworm consumption in cups with blueberry stems was nearly 100% after 48 h. In another experiment, we found fewer blueberry maggot pupae in arenas with a *Pterostichus melanarius* (Illiger) or *Harpalus rufipes* (DeGeer) (Coleoptar: Carabidae) beetle than in those without a beetle; however, soil moisture did not significantly affect pupal numbers even though maggots pupate more shallowly in wet than dry soil. Using PCR to analyze predator gut contents is becoming an important tool for studying biological control. A ~600 bp fragment of the cytochrome oxidase I (COI) mtDNA was sequenced and species-specific primers amplifying 207 bp for spanworm and 279 bp for flea beetle were designed and tested. *Carabus nemoralis*, *P. mutus*, and *P. lucublandus lucublandus* were killed at successive time intervals after feeding on a spanworm or flea beetle larva in the laboratory. Initial analysis showed detection half-lives (50% of samples testing positive) for pest DNA in the guts of beetles were 6-12 h, and detection was 0-10% after 48 h. Beetles were collected from two lowbush blueberry fields in spring 2012, and PCR procedures to detect spanworm and flea beetle consumption are in progress. With these results future experiments will be able to better address how beetle abundance and diversity affect pest density and whether management should be altered to conserve these predators.

# Impact of plant structural complexity and extraguild prey density on intraguild predation

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A number of ecological and abiotic factors determine the nature and frequency of intraguild predation (IGP), and thus the potential of IGP to interfere with biological control of arthropod pests. We examined the effect of plant structural complexity and extraguild prey density, and their interactions, on the occurrence of IGP between *Harmonia axyridis* (Pallas) and *Propylea quatuordecimpunctata* (L.) (Coleoptera: Coccinellidae). The theory predicts that IGP levels would increase with a decrease of both factors. We conducted a factorial experiment in an open soybean field into which coccinellid larvae were introduced in experimental plots for a period of five days. We tested two levels of aphid density, low (~100 aphids per plot) and high (~1000 aphids per plot), and two levels of plant structural complexity, low by removing half of the branches from the soybean plants and high by leaving plants intact. We used species-specific molecular markers to detect the presence of *P. quatuordecimpunctata* in the digestive tract of *H. axyridis*. Molecular gut-content analysis of *H. axyridis* revealed that rates of IGP were higher (≈ 20%) at low aphid density than at high aphid density (< 6%). Increased plant structural complexity had a negative influence on aphid density, but only at low aphid density treatments (reduction of 5% vs. 29% at high and low plant complexity, respectively). However, plant structure did not impact the frequency of IGP (12% and 13% at low and high complexity, respectively). According to the existing literature, this study demonstrates that IGP is amplified at low extraguild prey density. However, no relationship was found between IGP and plant structural complexity, perhaps as a consequence of the nature of the habitat structure we modified in our experiment.

# Do parasitized coccinellids contribute to aphid biological control?

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We have recently reported that females of the pink spotted ladybeetle, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) have the capacity to recover from parasitism by *Dinocampus coccinellae* Schrank (Hymenoptera: Braconidae). This outcome is extremely atypical since, by definition, parasitoids kill their hosts. Following parasitoid emergence, adult coccinellids are able to resume feeding and even successfully reproduce. The objective of the present study was to examine the efficacy of parasitized ladybeetles as biological control agents of aphids. We measured the voracity, fecundity and longevity of *C. maculata* females in the laboratory during and following parasitism by *D. coccinellae*. Over 80 % of the ladybeetles survived parasitism and were subsequently able to feed and reproduce. During parasitoid larval development and following parasitoid emergence, parasitized ladybeetles fed approximately 50% less than unparasitized individuals. Furthermore, they entered a state of paralysis and stopped feeding for a period of 10-15 days during parasitoid pupation. Coccinellids did not reproduce during parasitoid development, but started to lay eggs a few days after they recovered from parasitism. Their fecundity was then similar to unparasitized ladybeetles (5.3 ± 2.7 eggs vs. 6.7 ± 4.4 eggs, respectively). In addition, they lived longer than unparasitized ladybeetles (149.0 ± 48.1 days vs. 116.0 ± 27.6 days, respectively). Even if parasitized *C. maculata* live longer than unparasitized conspecifics, their voracity and reproductive output are significantly reduced over their entire life. However, from a biological control perspective, it appears that they are still capable of contributing to the reduction of aphid populations.

# Predatory potential of *Hippodamia convergens* and *Harmonia axyridis* on *Aphis gossypii* on okra

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A major problem for farmers is the occurrence of arthropod pests that cause economic loss of up to 30% in okra production. Besides the direct reduction on productivity, insects and mites are vectors of diseases and affect the quality of the product. Biological control through the action of insect predators is an efficient technology that can be used against aphids. In this study, we determined the ability of the coccinellids *Hippodamia convergens* Guérin-Méneville and *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) reduce the population density of *Aphis gossypi* Glover (Hemiptera: Aphididae) in okra plants under greenhouse conditions. Okra plants were cultivated in polyethylene pots of 5.0 kg capacity being protected by a cage covered with voile. After 20 days from seedling emergence each plant was infested with 100 adults of *A. gossypii*. Two days later the number of aphids per plant was evaluated and afterwards the predators were released. During periods of one, two and five days after the release of the coccinellids, we determined the number of aphids per plant. The following treatments were considered: T1- one adult of *H. axyridis* and *H. convergens*, T2- one 4th instar larvae of *H. axyridis*; T3- one adult and one 4th instar larvae of *H. axyridis* and T4- plant with aphids only. A randomized complete block design with five replicates was used. Data were subjected to analysis of variance, and means were compared by the Tukey's test at 5% significance. After one day of the release of coccinellids, it was found that *H. axyridis* and *H. convergens* reduced 52.8% the population density of *A. gossypii*. Two and five days after the release the largest percentage of population reductions of *A. gossypii* were observed in okra plants that received the simultaneous release of larvae and adults of the predators, compared to plants that received only 4th instar larvae of the coccinellids.

# Session 15: Consequences of reduced-impact pesticides for biological control agents and top-down suppression of arthropods in managed landscapes

## *Diglyphus isaea* for leafminer management and compatible alternatives for the management of other pests on Gerbera daisies

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*Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) is the most important arthropod pest on gerbera daisies grown under greenhouse conditions. The parasitoid *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) is effective in managing leafminer infestations. However, other pests, especially spider mites, can infest crops and must be managed. This discussion addresses management alternatives, for secondary pests, which allows the continued use of *D. isea* for leaminer control.

# Effects of a bifenthrin-treated net on the natural enemies *Aphidius colemani* (Haliday) and *Adalia bipunctata* L. in a cucumber crop in Central Spain. Semi-field experiments.

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Pest management practices that rely on pesticides are growing increasingly less effective and environmentally inappropriate in many cases, and the search for alternatives is under focus nowadays. Exclusion of pests from the crop by means of pesticide-treated screens can be an eco-friendly method to protect crops, especially if pests are vectors of important diseases. The mesh size of nets is crucial to determine if insects can eventually cross the barrier or exclude them because there is a great variation in insect size depending on the species. Long-lasting insecticide-treated (LLITN) nets, factory pre-treated, have been used for years to fight against mosquito vectors of malaria and are able to retain their biological efficacy under field for 3 years. In agriculture, treated nets with different insecticides have shown efficacy in controlling some insects and mites, so they seem to be a good tool in helping to solve some pest problems. However, treated nets must be carefully evaluated because can diminish air flow, increase temperature and humidity and decrease light transmission, which may affect plant growth, pests and natural enemies. As biological control is considered a key factor in IPM nowadays, the potential negative effects of treated nets on natural enemies needs to be studied carefully.

In this work, the effects of a bifentrhin-treated net (3 g/Kg) (supplied by Intelligent Insect Control, IIC) on natural enemies of aphids were tested on a cucumber crop in Central Spain in autumn 2011. The crop was sown in 8 x 6.5 m tunnels divided in two sealed compartments with control or treated nets, which were simple yellow netting with 25 mesh (10 x 10 threads/cm2; 1 x 1 mm hole size). Pieces of 2 m high of the treated-net were placed along the lateral sides of one of the two tunnel compartments in each of the 3 available tunnels (replicates); the rest was covered by a commercial untreated net of a similar mesh. The pest, *Aphis gossypii* Glover (Hemiptera: Aphididae), the parasitoid *Aphidius colemani* (Haliday) (Hymenoptera: Braconidae) and the predator *Adalia bipunctata* L. (Coleoptera: Coccinellidae) were artificially introduced in the crop. Weekly sampling was done determining the effects of presence or absence of the pest and the natural enemies (NE) in the 42 plants/compartment as well as the number of insects in 11 marked plants. Environmental conditions (temperature, relative humidity, UV and PAR radiation) were recorded.

Results showed that when aphids were artificially released inside the tunnels, neither the number/plant nor their distribution was affected by the treated net. A lack of negative effect of the insecticide-treated net on natural enemies was also observed. *Adalia bipunctata* did not establish in the crop and only a short term control of aphids was observed one week after release. On the other hand, *A. colemani* did establish in the crop and a more long-term effect on the numbers of aphids/plant was detected irrespective of the type of net.

# Side effects of modern pesticides on adults of the predatory mite *Amblyseius swirskii* under laboratory conditions

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*Amblyseius swirskii* (Athias-Henriot) (Mesostigmata: Phytoseiidae) is a polyphagous predatory mite which feeds on pollen and small arthropod prey such as whiteflies, thrips and mites. This species is widely used in IPM programs in greenhouses, for its success, it is essential to obtain information about the non target effects of the pesticides currently used in those crops where the mite is released. This work describes a laboratory contact residual test for evaluating lethal (mortality after 72 h exposure to fresh residues) and sublethal effects (fecundity and fertility of the surviving mites) of eleven modern pesticides to adults of *A. swirskii*. Spiromesifen is a lipogenesis inhibitor; flonicamid a selective feeding inhibitor with a mode of action not totally known; flubendiamide a modulator of the rhyanodin receptor; sulfoxaflor has a complex mode of action not totally ascertained; metaflumizone is a voltage dependent sodium channel blocker; methoxyfenozide is an IGR; spirotetramat inhibits lipids; abamectin and emamectin activate the Cl- channel; spinosad is a neurotix naturalyte; and deltamethrin a pyrethroid used as a positive standard. Selected pesticides are effective against different key pests present in horticultural crop areas and were always applied at the maximum field recommended concentration in Spain if registered, or at the concentration recommended by the supplier.

Out of the tested pesticides, spiromesifen, flonicamid, flubendiamide, sulfoxaflor, metaflumizone, methoxyfenozide and spirotetramat were harmless to adults of the predatory mite (IOBC toxicity class 1). The rest of the pesticides exhibited some negative effects: emamectin was slightly harmful (IOBC 2), deltamethrin moderately harmful (IOBC 3) and spinosad and abamectin harmful (IOBC 4). Further testing under more realistic conditions is needed for those pesticides having some harmful effect on the mite prior deciding their joint use or not.

# Toxicity of sulfur, mineral oil, abamectin and pyridaben on *Neoseiulus californicus*

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Toxicity of abamectin, mineral oil, sulfur and pyridaben was evaluated on the predatory mite *Neoseiulus californicus* (McGregor) (Mesostigmata: Phytoseiidae), a predator of *Oligonychus yothersi* (McGregor) (Trombidiformes: Tetranychidae), an important pest of avocados in Chile. In a first experiment, pesticides were applied to mobile stages or eggs of *N. californicus* on avocado leaf disks. While at the start mortality of mobiles was lower with mineral oil (74%) and sulfur (62%), it increased over time (80%), and with abamectin and pyridaben it was close to 100%. Hatching of *N. californicus* eggs with abamectin, with distilled water and in the control reached almost 75%, and it decreased with pyridaben, sulfur (63%) and mineral oil (24.5%). A second experiment evaluated the effect of sub-lethal doses of mineral oil and sulfur on voracity, fecundity, and fertility of *N. californicus*. While consumption in the control treatment was 13.7 *O. yothersi* (out of 24 offered), it decreased with sulfur (6.7) and mineral oil (2). The average eggs/female laid in the control was 11.3, decreasing with sulfur (3) and mineral oil (5.8). Egg hatching was statistically similar in the control (76.3%) and sulfur treatment (59%) and significantly lower with mineral oil (24%). A third experiment evaluated mortality of mobile stages of *N. californicus* caused by exposure to treated avocado leaves 7, 10, 17, 21, and 28 days after pesticides application. At day 7, mortality reached more than 80% with abamectin, mineral oil, and pyridaben, and only 55% with sulfur. At day 10 mortality by mineral oil decreased to 48%, remaining high with abamectin (90%) and pyridaben (66.7%). At day 17, only pyridaben differed from the other treatments (71.7% mortality), and mineral oil and sulfur decreased mortality to 23.4%. At days 21 and 28, mortality in all treatments were similar and lower than 18%. Mortality in the control always remained between 5 and 11.7%. These results suggest that these miticides can be toxic to this predator, therefore its release must be compatible with the direct or residual effect of pesticides. Also, mineral oil and sulfur can decrease reproductive rate and fertility of *N. californicus*, which may restrict their use for integrated management programs.

# Evaluation of neem-based formulations on *Harmonia axyridis* in okra

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Neem products extracted from *Azadirachta indica* A. Juss (Meliaceae) can be used to control agricultural pests. This study evaluated the effect of neem on larvae of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in okra, *Abelmoschus esculentus* (L.) Moench (Malvaceae), under greenhouse conditions. Single okra plants were grown in pots and protected by cages with voile. When the plants reached a height of 20 cm they were sprayed with neem formulations according to the following treatments: 1) commercial oil-based formulation of 0.5%, 2) commercial oil-based formulation of 1%, 3) aqueous leaf extract, 4) aqueous seed extract and, 5) control - plants sprayed with distilled water. We used a completely randomized design with 10 replications. After an hour of spraying, 100 adults and nymphs of the aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) were released per plant. Subsequently, one one-day-old 1st instar larva of *H. axyridis* was introduced on each plant. Aphids were replaced when necessary, and through daily observations the percent of larvae surviving was determined. After eight days from the beginning of the study, it was observed that about 70% of the *H. axyridis* larvae had abandoned the plants treated with the neem formulations, the remaining larvae did not survived. These results indicate that, although it is recognized that neem-based formulations can control agricultural pests, the repellent action and mortality observed in larvae of *H. axyridis* in okra plants demonstrated that applying this vegetable insecticide would decrease the efficiency of biological control provided by *H. axyridis* and even other coccinellid species.

# Survival of the parasitoid wasps *Cotesia glomerata* and *Copidosoma* sp. to natural products

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The use of natural products to control pest insects grows every year. However, there is still a lack of information on the use of these products. Among the missing information is highlighting the selectivity to natural enemies, for example, the parasitoid wasps that are important biological control agents for several insect pests. This study aimed to evaluate the effect of natural products on the survival of *Cotesia glomerata* and *Copidosoma* sp. The bioassays were carried out at the Department of Entomology, at Federal University of Lavras, MG, Brazil. Extracts of the natural products tobacco, pepper, citronella, garlic and mix (a mixture of all extracts) at a concentration of 8% were evaluated. As a positive control we used distilled water and as a negative control the insecticides chlorfenapyr (Pirate® 0.24 g a.i. L-1) and etofenproxi (Safety®; 0.18 g a.i. L-1) applied to *C. glomerata* and *Copidosoma* sp., respectively. The insecticides were sprayed in high doses recommended for the control of *Ascia monuste orseis* in brassica crops and for control of *Neoleucinodes elegantalis* in tomato crops, the target pests of the parasitoid wasps. The bioassays were carried out in a completely randomized design with 30 replications. Each replication consisted of a parasitoid, aged up to 24 hours, individualized in 5 cm diameter Petri dishes containing a sprayed cabbage leaf disc (bioassay *C. glomerata*) and a sprayed tomato leaf disc (bioassay *Copidosoma* sp.). The spray was applied via a Potter tower. The parasitoids were exposed to the treatments 1h after spraying. The Petri dishes were kept in a room at 25 ± 2ºC, RH 70 ± 10% and a photophase of 12 hours. The survival of the parasitoids was assessed 2, 6, 12, 24, 48, 72 and 96h after exposure to these treatments. The time mortality data were submitted to survival analysis by the Kaplan-Meier method. Survival analysis of the parasitoids when exposed to dry residues of the products indicated significant differences between products for both *C. glomerata* (Log-rank test, x2 = 57.61, df = 6, P <0.001) and *Copidosoma* sp. (Log-rank test, x2 = 220.76, df = 6, P <0.001). Support: CNPq, CAPES and FAPEMIG.

# Selectivity of compounds used in corn crops in Spain on larvae of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae)

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Green lacewings are predatory insects found in several agroecosystems and are commonly used in biological control programs for pests. *Chrysoperla carnea* is commonly found in corn fields in Spain, where it feeds on various arthropod pests. Thus, the aim of this work was to evaluate the selectivity of compounds used in corn crops on 3rd instar larvae of *C. carnea*. The compounds, used in their higher dosages recommended by the manufacturers, were deltamethrin (1.5% DECIS® EW, pyrethroid), chlorpyrifos (CHAS® 48% EC, organophosphate), λ-cyhalothrin (KARATE® 10% WG, pyrethroid), abamectin (APACHE® 1.8% EC, avermectin) hexythiazox (PROFILE® 10% WP, carboxamide) and pendimethalin (PENDALIN® 33% EC, dinitroaniline). The control treatment consisted only of distilled water. To perform the experiment, glass plates were treated with the compounds via Potter tower and then 3rd instar larvae of *C. carnea* were kept in contact with their contaminated surfaces until the formation of pupae. The bioassay was carried out in a room at 25 ± 2°C, RH 70 ± 10% and 16h photophase. A completely randomized design was used, with seven treatments and six replications, each plot consisted of seven larvae of *C. carnea*. The effects of the compounds on larval mortality, the duration of the 3rd instar and the pupal stage, the percent pupation, fecundity and fertility of eggs produced by adults reared from treated larvae were evaluated. Due to the total effect, each product was assigned to a toxicity class according to the IOBC guidelines. Therefore, chlorpyrifos was harmful (class 4); deltamethrin was moderately harmful (class 3), λ-cyhalothrin, abamectin and hexythiazox were slightly harmful (class 2) and pendimethalin was harmless (class 1). Due to the toxicity of chlorpyrifos and deltamethrin demonstrated in the laboratory, it is recommended that further studies be conducted to test them in greenhouse and field conditions. Since λ-cyhalothrin, abamectin, hexythiazox and pendimethalin were slightly toxic to the predator under extreme exposure conditions, these compounds can be recommended in integrated pest management programs in corn crops aiming to preserve this species of green lacewing. Support: CNPq, CAPES and FAPEMIG.

# Inoculative release of a beneficial wasp egg parasitoid, *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) to supplement natural wasp populations for control of *Helicoverpa* in Northern New South Wales field crops

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In northern NSW, *Helicoverpa* spp. (Lepidoptera: Noctuidae) is a seasonal pest of several winter and summer crops. Chickpeas, canola, sorghum and maize are particularly vulnerable to attack over economic thresholds on the Liverpool Plains and the primary method of control is pesticide application. Similarity the bulk of cotton grown on the Liverpool Plains is genetically modified to kill *Helicoverpa* larvae, and Bt resistance by *Helicoverpa* is escalating, resulting in late season *Helicoverpa* pesticide application. Alarmingly little attention has been given to the natural enemies of *Helicoverpa*, which have been shown in other parts of Australia and worldwide to be significant to any IPM program. Surveys by Carr et al. (2009) on naturally occurring populations of *Trichogramma* on the Liverpool Plains in crop and in non-crop habitats has concluded that low background Trichogramma populations are prevalent with peak populations active at the end of January in cotton, sorghum and late season maize. To be more effective as a natural enemy, we demonstrate here that *Trichogramma* populations can be successfully increased with early season spring inoculative releases in *Helicoverpa* prone crops. Subsequently we propose that such increases benefit adjoining summer crops.

# Session 16: Pest Management for an Urbanizing World: Arthropod Biological Control in Ornamental Landscapes

## Biological control of *Protopulvinaria pyriformis* (Hemiptera: Coccidae) in urban landscapes. How to overcome its phenology and defenses?

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*Protopulvinaria pyriformis* (Cockerell) (Hemiptera: Coccidae) is an invasive soft scale of Neotropical origin. It is widely distributed in America, southern Africa and the Mediterranean Basin, where it is a pest of tropical and subtropical fruit trees and ornamental plants. In Spain and Greece it causes severe damage on laurel trees Laurus nobilis L. (*Lauraceae*) in urban landscapes. *Metaphycus helvolus* (Compere) (Hymenoptera: Encyrtidae) is the main parasitoid of *P. pyriformis* in both countries but its biological control remains unsatisfactory. Therefore, in this work we studied the interactions between *P. pyriformis* and *M. helvolus* under field and laboratory conditions to understand the biological control failure. *Metaphycus helvolus* populations were highly influenced by *P. pyriformis* phenology. Parasitoid abundance decreased in summer due to the scarcity of suitable hosts and the presence of small scale sizes that produced a male-biased offspring. Moreover, *M. helvolus* was not able to overcome different defense strategies of the soft scale: 1) *P. pyriformis* did not encapsulate *M. helvolus* eggs, but high mortality rates occurred during the parasitoid prepupal stage; 2) *M. helvolus* could not use *P. pyriformis* honeydew as a carbohydrate source to increase its life span; and 3) *P. pyriformis* defended actively against *M. helvolus* and it frequently avoided oviposition. The latter is a novel host defense in soft scales. Finally, we discuss how to overcome these constraints to improve the biological control of *P. pyriformis* in urban landscapes.

# Effects of *Protopulvinaria pyriformis* host size on *Metaphycus helvolus* sex allocation patterns

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*Protopulvinaria pyriformis* (Cockerell) (Hemiptera: Coccidae) is a key pest of laurel trees *Laurus nobilis* L. (Lauraceae) in the Mediterranean basin. *Metaphycus helvolus* (Compere) (Hymenoptera: Encyrtidae) is its main parasitoid in some countries of this region. However, biological control is insufficient, likely because the parasitoid population decreases when scales are small. The aim of this work was to determine whether the sex allocation patterns of *M. helvolus* may lead to a reduction of its population levels. For this, we analyzed field data and carried out several laboratory assays. Our field data showed that the sex ratio pattern of *M. helvolus* was host size dependent and it allocated more females in larger scales. The threshold above which *M. helvolus* allocated only female offspring was rather absolute than relative under both field and laboratory conditions and the secondary sex ratio became female biased on scales larger than 1.1 mm. As a consequence of this behavior, *M. helvolus* populations strongly decreased during summer when only small scales were present in the field, resulting in unsuccessful biological control. Moreover, these findings have important implications for improving *M. helvolus* mass-rearing and application of augmentative biological control to *P. pyriformis*.

# Impacts of urbanization on ground beetle communities

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Urbanization brings radical changes to landscapes as natural communities of forests, deserts, and prairies transform into cities and suburbs with attendant hardscape, degraded soils, elevated temperatures, pollutants, and contrived biodiversity of plant and animal communities (McDonnell and Pickett 1990, Faeth et al. 2005, Raupp et al. 2010). Urbanization in many parts of the world shows no indication of slowing. For example urban areas in the United States are predicted to increase over current levels by 74% - 164% by 2100 (Bierwagen et al. 2010). In many locations urban areas are juxtaposed with natural ones, thereby creating a gradient where patterns of biodiversity and ecosystem processes can be studied (McDonnell and Pickett 1990, McIntyre 2000, McKinney 2008). Recent articles have examined ecological responses of herbivorous arthropods along urbanization gradients and found several taxa of arthropods achieving greater abundance in urban compared to natural settings (Raupp et al. 2010, 2012). One mechanistic explanation for these results was thought to be a loss of top-down regulation with increasing levels of urbanization. Ground beetles are a diverse taxon whose members engage in varying levels of herbivory, omnivory, and predation. They have been well-studied along gradients ranging from cities to rural areas. We attempted to elucidate the overall impacts of urbanization on ground beetles (Coleoptera: Carabidae). Through an extensive literature search, we compiled data including 658 species records of 327 carabid species (Martinson and Raupp, in review). With this data we employed meta-analysis to estimate the effect of urbanization on carabid species richness, the total abundance of ground beetles (assemblage abundance), and individual species abundances. Next, we examined how factors such as the human population size of a city and the distance between study sites (rural vs city) could be used to understand variation in species richness and assemblage abundance. We also determined the importance of a suite of life history traits including habitat affinity, body size, wing morphology, and trophic position on the responses of species to urbanization. By employing model selection based on information theoretic measures (Burnham and Anderson 2002) we were able to identify characteristics of cities and species that best explained the variation in the data set. Finally, because we were able to find multiple studies for widespread species, we examined the consistency of their responses to urbanization among cities. With respect to changes in ground beetle species richness in urban compared to rural sites, there was a significant reduction. However, with respect to the total abundance of the ground beetle community, there was no difference between rural and urban sites. Human population size of a city, distance between rural and urban sites, and duration of the study had no effect on the number of species or total number of individuals of carabids. By contrast, variation in ecological species traits did explain reduction in individual species abundances along the urbanization gradient. A model comprised of beetle size, habitat affinity, and their interaction explained the most observed variation in abundance. Even though experimental samples of beetles were taken in forested habitats in both cities and rural areas, large, forest-specialist species retained only 6.4 % of their rural abundances in cities. Our suite of ecological traits explained significant variation in species responses, but much variability among studies, species, and populations could not be explained by our analysis. To further identify sources of this variation and the mechanisms underlying ground beetle declines in cities will require targeted empirical or experimental studies. Reductions in species richness and abundance suggest that some taxa may be harbingers environmental degradation in cities with a concomitant loss of some ecological services such as biological control of pests.

## References

Bierwagen, B. G., D. M. Theobald, C. R. Pyke, A. Choate, P. Groth, J. V. Thomas, and P. Morefield. 2010. National housing and impervious surface scenarios for integrated climate impact assessments. P. Nat. Acad. Sci. USA 107: 20887–20892.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. 2nd Edition. Springer, New York, New York, USA.

Faeth, S. H., P. S. Warren, E. Shochat, and W. A. Marussich. 2005. Trophic dynamics in urban communities. BioScience 55: 399-407.

Martinson, H. and M.J. Raupp. In review. A meta-analysis of the impacts of urbanization on ground beetle communities.

McDonnell, M. J. and S. T. A. Pickett 1990. Ecosystem structure and function along urban-rural gradients: an unexploited opportunity for ecology. Ecology 71: 1232-1237.

McIntyre, N.E. 2000. Ecology of urban arthropods: a review and call to action. Ann. Entomol. Soc. Amer. 93: 825–835.

McKinney, M. L. 2008. Effects of urbanization on species richness: a review of plants and animals. Urban Ecosyst. 11: 161–176.

Raupp, M. J., P. M. Shrewsbury, and D. A. Herms. 2010. Ecology of herbivorous arthropods in urban landscapes. Ann. Rev. Entomol. 55: 19-38.

Raupp, M. J., P. M. Shrewsbury, and D. A. Herms. 2012. Disasters by design: outbreaks along urban gradients, pp. 313–340 *In* P. Barbosa, D. Letourneau, and A. Agrawal, (eds.) Insect outbreaks revisited. Wiley-Blackwell, West Sussex, UK.

# Session 17: Evaluation and practical application of parasitoids and predators of the tomato leaf miner *Tuta absoluta*

## Rapid detection of key endoparasitoids in *Tuta absoluta*, *Symmetrischema tangolias* and *Phthorimaea operculella* using multiplex PCR

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Parasitoids play an important role as antagonists of lepidopteran pests. However, tracking host-parasitoid interactions with conventional methods such as host dissection and the rearing of parasitoids is difficult and time consuming. Molecular methods can overcome these hurdles: collected hosts can be stored in ethanol or in a freezer, and therefore no specific rearing facilities are needed. The samples can be analysed at any time for the presence of parasitoid DNA independent of the life cycles of hosts and parasitoids. Furthermore, potential data loss due to animals dying during rearing is prevented. Here we present a rapid molecular system which allows, within one reaction, detecting the five common parasitoid species *Apanteles subandinus* Blachard (Hymenoptera: Braconidae), *Copidosoma koehleri* Blanchard (Hymenoptera: Encrytidae), *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae), *Orgilus lepidus* Musebeck and *Dolichogenidea gelechiidivoris* (Nixon) (Hymenoptera: Braconidae). While the latter is a common parasitoid of the tomato leaf miner *Tuta absoluta* (Meyrick), the other parasitoid species regularly attack the Andean potato tuber moth *Symmetrischema tangolias* (Gyen) and the potato tuber moth *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). Additionally, our assay also identifies the three moth species, which is helpful when investigating early instar caterpillars. To reduce screening effort when low parasitism rates are expected, we will also evaluate a sample pooling approach using this multiplex PCR system.

# Evaluation of biological control strategies of *Delia platura* (Meigen), under laboratory conditions

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Spinach, *Spinacia oleracea* L. (*Amaranthaceae*) is a vegetable widely consumed in Colombia. *Delia platura* (Meigen) (Diptera: Anthomyidae) is one of the key pests in spinach. The damage is caused by the larvae, which bore into the leaf petiole and stem creating injuries to the growing points. The management of this pest has relied upon the inappropriate use of chemical pesticides, with the corresponding impacts on the environment. Separate evaluations were carried out to assess the generalist parasitoid of Diptera, *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) and five entomopathogens for control of *D. platura* under laboratory conditions. Procedures to field capture and breed *D. platura* were developed. For the parasitoid evaluation, the experimental unit consisted of a parasitoid female individual and five pupae of *D. platura* isolated in a Petri dish. Ten repetitions were included, and as a test control the parasitism on pupae of *Musca domestica* L. (Diptera: Muscidae) was also evaluated. The entomopathogens and experimental doses were: no control, *Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae) DSM-14943 (1.0 g l-1), *B. bassiana* strain GHA (1.0 cc l-1), *Entomophthora virulenta* Hall et Dunn (Entomophthoraceae) (1.0 cc l-1), *Metarhizium anisopliae* (Metschnikoff) Sorokin (Clavicipitaceae) DSM-15168 (2.0 cc l-1) and *Bacillus thuringiensis* Berliner servoar. *israelensis* (Bacillaceae) (1.0 g l-1). The treatments were applied to the last instar larvae of *D. platura*. For this evaluation, a Completely Randomised Design considering six treatments with five replications was applied. The experimental unit consisted of five larvae inside a Petri dish containing spinach biomass and soil. Mortality was registered on a daily basis. The bioassays were kept inside a climate controlled room at 20±1ºC, 70±10% R.H. and 12h L:D. The mortality was determined through the Henderson-Tilton test. The parasitism of *P. vindemmiae* on *D. platura* was 62±31.9% while on *M. domestica* was 40±29.8%, not showing significant differences according to the Mann-Whitney test (p-value=0.15222). The control efficacies for *B. bassiana* GHA, *B. bassiana* DSM-14943, *B. thuringiensis* subsp. *israelensis*, *M. anisopliae* DSM-15168 and *E. virulenta* were 52.4%, 47.6%, 42.9%, 38.1% and 23.8%, respectively. The bioassay showed significant differences through the Kruskal-Wallis test (p-value=0.006337). The results confirmed the parasitism of *P. vindemmiae* against *D. platura* and the possibility to include different biological agents to control this arthropod.

# Ecotoxicity of insecticides of frequent use in tomatoes on *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae)

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Since *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) was detected in 2006 as a new pest in tomato crops in Spain, several natural enemies have been reported to control this pest. In biological control programs, the native parasitoid *Trichogramma achaeae* Nagaraja & Nagarkatti (Hymenoptera: Trichogrammatidae) is used against *T. absoluta*. However, the most common control practice is based on use of pesticides, and in the context of Integrated Pest Management (IPM) programs, knowledge on the activity of insecticides towards beneficial insects is needed for its joint use. In this work, we evaluated lethal and sublethal effects of insecticides commonly applied on tomato crops on adults of *T. achaeae*. Pesticides were sprayed on tomato plants or *T. absoluta* eggs till run off at their maximum field recommended concentration. Mortality was scored after 24, 48 and 72 hours, as well as beneficial capacity and percentage of emergence.

# Session 18: Compatibility of transgenic insecticidal crops with biological control

## Compatibility of transgenic legumes and parasitoids to control bruchids (Coleoptera: Bruchidae)

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Bruchid beetles (Coleoptera: Chrysomelidae) are the most important pests of the stored seeds. Bruchid infestations are difficult to manage by conventional methods and despite intensive conventional breeding efforts, bruchid-resistant varieties of chickpea, *Cicer arietinum* L. and cowpea, *Vigna unguiculata* (L.) Walp. (*Fabaceae*) have not been achieved. In contrast, it is long known that seeds of the common bean are immune to important bruchid pest species, including *Callosobruchus chinensis* L. and *C. maculatus* (Fabricius). An α-amylase inhibitor (αAI-1) from the common bean, *Phaseolus vulgaris* L. (*Fabaceae*), has been identified as a major source of resistance in bean seeds. Genetic engineering has been used to transfer this resistance factor into other legumes, including chickpea and cowpea. The presence of αAI-1 in transgenic seeds significantly increases their resistance to sensitive bruchid species. In contrast, *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman), originally pests of the common bean, are not affected by αAI-1. A powerful approach to control also such tolerant species might be the combination of αAI-1 transgenic seeds with hymenopteran parasitoids. However, parasitoid larvae or host-feeding females might therefore get in contact with the inhibitor when attacking resistant bruchid larvae that have ingested αAI-1. However, whether bruchid parasitoids rely on α-amylases for carbohydrate digestion and whether they are significantly exposed and harmed by the inhibitor has so far not been investigated. We have developed a conceptual model describing a pathway on how the presence of αAI-1 in GM legume seeds might negatively interfere with the biological control services provided by bruchid parasitoids. The steps of our model include the characterization of α-amylase activity in parasitoid extracts, the in vitro sensitivity of the digestive enzyme to αAI-1, as well as the assessment of the hazard and exposure to the plant-expressed αAI-1 in tritrophic experiments.

We have characterized the α-amylase activity in five different hymenopteran parasitoid species commonly used to control bruchids: *Anisopteromalus calandrae* (Howard), *Dinarmus basalis* (Rondani), *Lariophagus distinguendus* (Förster) (Hymenoptera: Pteromalidae), *Eupelmus vuilleti* (Crawford) (Hymenoptera: Eupelmidae) and *Heterospilus prosopidis* Viereck (Hymenoptera: Braconidae). We found α-amylase activity in females and larvae of all parasitoid species, suggesting that both larvae and females rely on α-amylases for carbohydrate digestion. In vitro inhibition studies have confirmed that α-amylase activity of both larvae and females of all parasitic wasps are strongly susceptible to αAI-1. We could not detect an adverse effect on the parasitoid *D. basalis* when attacking larvae of the αAI-1 tolerant bruchid *Z. subfasciatus* developing in αAI-1 transgenic cowpea seeds. Whether αAI-1 is transferred in detectable amounts from the seeds through the host to the parasitoids is currently under investigation.

# Session 19 a): Classical Biological Control in crops and others

## Invasive exotic pests in European vineyards: prospects for biological control?

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The rise of global trade since the 1950s has greatly increased the risk of introduction and establishment of potential grapevine pests from one region to another. All groups of arthropods are subject to be introduced, but some seem to be more prevalent such as leafhoppers, and moths. Since the end of the 19th century, European viticulture has been under pressure from exotic pest species, starting with the massive negative impact of the North American Phylloxera. This has been in addition to the threat of indigenous pests, like the grapevine tortricids. This paper examines a dozen of known exotic insect pests reported from grapevines in Europe in the last 50 years; most of them originating from North America. Each species is presented with biological distribution, and data on potential or effective management. These pests are the ladybird beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), *Jacobiasca lybica* (Bergenin and Zanon), *Erasmoneura vulnerata* (Fitch), *Scaphoideus titanus* Ball, (Hemiptera: Cicadellidae) *Metcalfa pruinosa* (Say) (Hemiptera: Flatidae) , *Acanalonia conica* (Say) (Hemiptera: Acanaloniidae), *Stictocephala bisonia* (Kopp and Yonke) (Hemiptera: Membracidae), *Aphis illinoisensis* Shimer (Hemiptera: Aphdidiae); the moths *Hyphantria cunea* Drury Lepidoptera: Arctiidae), *Phyllocnistis vitegenella* Clemens (Lepidoptera: Gracillariidae); the thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) ; and the fly *Janetiella oenophila* (Haimhoffer) (Diptera: Cecidomyiidae). A proactive management strategy for exotic pests is presented, using the leafhopper *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) as a model. At present, this species has not been introduced into Europe, but it is nonetheless considered as an important potential threat to European viticulture. A mirror analysis will be given with other grapevine pests worldwide originating from Eurasia.

# Status of biological control of red imported fire ants with phorid flies in Louisiana

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The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), invaded the United States from South America more than 75 years ago. It was first discovered in Louisiana in the early 1950s. Because of its aggressive behavior and lack of natural enemies, the red imported fire ant has expanded its range into at least 13 states and Puerto Rico. It is present throughout most of the southeastern United States, where it is considered an economically important pest. Because the red imported fire ant invaded the United States without its natural enemies, attention has recently focused on the potential for biological control by importing specialist parasitoids in the decapitating phorid fly genus, *Pseudacteon* (Diptera: Phoridae).

In Louisiana, research in the past 13 years has focused on the establishment of new phorid fly species, their spread, and studying their potential impact on red imported fire ants. Five phorid species: *P. tricuspis* Borgmeier, *P. curvatus* Borgmeier, *P. litoralis* Borgmeier, *P. obtusus* Borgmeier, and *P. cultellatus* Borgmeier were released. *Pseudacteon tricuspis* was released at seven locations with establishment confirmed at four locations. The first successful release of *P. tricuspis* was in 1999. Since 2005, three successful releases have been made with *P. curvatus*. A single unsuccessful release of *P. litoralis* was made in 2006. Releases were attempted in 2009 and 2010 for *P. obtusus* and in 2011 for *P. cultellatus*. Establishment of these two species was not confirmed.

In 2009, a survey was conducted from the spring to the fall in all Louisiana parishes to determine the distribution of *P. tricuspis* and *P. curvatus*. A total of 136 sites were sampled. Sampling sites were located on roadsides bordered by forests, agricultural fields or pastures. Ten mounds were sampled at each site. All flies were collected with an aspirator and brought to the laboratory for identification. Identified flies were sexed (*P. tricuspis*) and counted. In 2010, a follow-up survey was conducted in parishes where either or both fly species were missing. In collaboration with the USDA-ARS, collected phorid flies were tested for *Kneallhazia* (=*Thelohania*) *solenopsae* (Microsporidia: Thelohaniidae), another biological control agent of red imported fire ants.

In 2009, a four-year study was initiated to measure the potential impact of the two established phorid species on fire ants. Fire ant populations in the presence of *P. tricuspis* and *P. curvatus* were compared to fire ant populations without either phorid species. Phorid fly abundance and fire ant population size were measured in the spring and in the fall. The number of workers and presence or absence of brood was used to characterize fire ant populations using Harlan's population index rating system (PI).

# Potential of hymenopteran parasitoids for classical biological control of leafminer flies

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Leafminer flies are important agricultural pests worldwide. Since the mid-1970s the polyphagous New World species, *Liriomyza sativae* Blanchard, *L. trifolii* (Burgess) and *L. huidobrensis* (Blanchard) (Diptera, Agromyzidae) have been invading new geographical areas and have become a threat to horticultural crops in several countries of Asia and Africa. In Kenya, these invasive leafminer species are devastating pests with infestation between 10-80% and with *L. huidobrensis* as the most important species (80%). The diversity of parasitoids is low in all horticultural production systems in Kenya (4 species) and a rate of parasitism of <5% across all agroecozones and infested crops. Natural enemies are important in regulating Liriomyza leafminers in their native and invaded areas. Investigation at Peruvian highlands and coastal vegetable cropping systems yielded more than 60 hymenoptera parasitoids constituting a natural rich source of biological control. Three key endoparasitoids of *L. huidobrensis*: *Chrysocharis flacilla* (Walker) (Hymenoptera: Eulophidae), *Phaedrotoma scabriventris* Nixon (Hymenoptera: Braconidae) and *Halticoptera arduine* (Walker) (Hymenoptera: Pteromalidae) were identified. These parasitoids were found in wide ecological amplitude from the coastal to highland regions, which clearly indicate their adaptation to different climates. Because of their wide host plant and leafminer fly associations these species were selected as agents for classic biolioligcal control of *Liriomyza* spp. in Kenya. Aspects about distribution, seasonal phenology, host range and biology are discussed.

# Bio-control efficiency of two aquatic bugs against mosquito larvae *Culex quienquefasciatus* Say

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The paper incorporates the results of investigations on quantitative estimation of the predatory efficiency of two aquatic insects, *Diplonychus rusticus* (Fabricius) (Hemiptera: Belostomatidae) and *Ranatra filiformes* Fabricius (Hemiptera: Nepidae). The selected heteropterans are abundant in the rice field, pond and temporary pools of Assam, India. The predatory efficiency of the selected predators were made under laboratory conditions using the fourth instar larvae of *Culex quienquefasciatus* Say (Diptera: Culicidae) as prey. This mosquito transmits the diseases namely filariasis and encephalitis in human and animals. The destructive propensity in terms of Predatory Impact (PI) and Clearance Rate (CR) values varied between the predator species. Mean number of *C. quienquefasciatus* larvae killed by *D. rusticus* ranged between 18 to 34 larvae per day depending upon the predator and prey densities. In comparison, *R. filiformes* consumed the prey at the rate of 8 to 22 per day. The feeding rate of both the predators varied significantly between dark and light conditions. The results of the present investigation indicates the importance of aquatic bugs as natural mosquito regulators and possible use of these bugs as biological control agent against mosquito vector under agro-climatic condition of Assam, India.

# Impact of releasing the egg parasitoid *Telenemus busseolae* (Hymenoptera: Scelionidae) for control of sugarcane stalk borer *Sesamia* spp. in south west Iran: A three year experience

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Sugarcane is grown extensively throughout the world, and this crop is attacked by a variety of pests. Among them, stalk borers are a diverse group of species occurring in several genera, and are mainly polyphagous pests of gramineous crops including corn, sugarcane, rice, millet and sorghum. They cause economic loss in sugarcane and other crops through damage to stem and stalk by larval boring. Pink stem borers, *Sesamia* spp. (Lepidoptera: Noctuidae), are the most destructive pests of sugarcane in South west of Iran, where it has been managed largely through the planting of resistant cultivars, cropping practices and biological control. *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae) is the most important egg parasitoid of sugarcane stem borers in Iran, and this biological control agent can sometimes reduce the damage of moth borers in sugarcane cultivars. As establishment of insectarium for three years, We released large numbers of insectary-reared *T. busseolae* annually in 3 different periods (early April, mid June and mid September) to evaluate reduction of the damage of stalk borers in commercial fields. Our results showed that this egg parasitoid has potential for controlling stem borers and reducing damage below the economic injury level. The best results were obtained at harvest time when more than 90% of collected eggs were parasitized on sampled stalks (we select randomly 200 stalks for each 10 hectare-field). As a result, *T.busseolae* has a significant effect on moth borer eggs and can be an important part of borer reduction in sugarcane fields and is potentially a key factor in sugarcane IPM.

# Session 19 c): Conservation BC in crops and others

## Effect of flowering trap crops on insect pests and their natural enemies

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Indian mustard, *Brassica juncea* (L.) Czern, and yellow rocket, *Barbarea vulgaris* R. Br., (*Brassicacea*) have been proposed as trap crops for the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae). Flowers of these trap crops could also be used as a nectar source for insect feeding. Here we study the potential of flowering *B. juncea* and *B. vulgaris* to reduce the populations of *P. xylostella*; to attract aphidophagous syrphid flies; and to increase the populations of parasitoids of *P. xylostella* in cauliflower crops in Spain.

# Strawberries, alfalfa, and parasitoids: synergy in pest management

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Trap crops of alfalfa interspersed into organically produced strawberry fields grown along the central coast of California are being used to manipulate the spatial distribution of Lygus bugs (Hemiptera: Miridae), a key pest to this high value crop. Narrow strips of alfalfa are used to attract Lygus bugs out of, or away from, strawberries. Marking studies and season long monitoring of insect populations demonstrate that high spatial concentrations of Lygus bugs in the highly preferred host plant alfalfa have several effects. They can reduce the number of Lygus bugs feeding on strawberries and make their removal (via vacuum) more efficient. Secondly, high concentrations of Lygus bugs benefit the introduced Lygus bug parasitoid *Peristenus relictus* Loan (Hymenoptera: Braconidae). Like other specialized parasitoids, there is a density-dependent response by *P. relictus* to Lygus bug numbers. The clustered distribution of Lygus bugs nymphs provides pockets of high host densities that help elevate parasitism rates and subsequent numbers of *P. relictus* adults. The high numbers of parasitoids spill out and attack the remaining, much lower densities of Lygus bugs in adjacent rows of strawberries.

# Field boundary effect on the biological control of plant pests

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Field boundaries play an important agricultural role in providing a refuge for beneficial arthropods, enhancement of biological pest control, and and reduction of agrochemical drift. This study investigated whether different types of field boundary have different impacts on biological pest control in adjacent fields. Densities and diversity of selected predator guilds and the predation rates on a sentinel prey, eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), were compared between four field boundary types at a farm-scale. Arthropod predators studied were spiders, ground and rove beetles. Predator sampling and predation measures were made at four distances (0, 5, 20 and 40 m) from the field boundaries.

Herbaceous boundaries had a great impact on the predation rate on E. kuehniella eggs compared to field margins with trees. The latter were less favorable to the development of predators, particularly ground beetles. Distance from the field margins was found to not affect egg predation. However, predator abundance was influenced by distance to boundary. Beetles and spiders were more abundant near the boundary (0 and 5m). Although ground beetle diversity was affected by the boundary type, community composition likely depended on other landscape variables. This study suggests that predator arthropods and biological control of pests can be best maintained by managing simple, grassy field margins.

# Spontaneous weed strips associated with chili pepper agroecosystems promote the abundance and survival of aphid predators

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Habitat manipulation has long been used as strategy to enhance beneficial insects in agroecosystems. Spontaneous plant strips have the potential of supplying food resources to natural enemies, even when pest densities are low. However, in tropical agroecosystems there is a paucity of information pertaining to the resources provided by spontaneous weeds and their interactions with natural enemies. In this study we evaluated: 1) whether spontaneous weeds within chili pepper fields affect the diversity and abundance of aphidophagous species, 2) whether there are direct interactions between spontaneous weed and aphidophagous arthropods, and 3) the importance of spontaneous weed floral resources for survival and reproduction of a native and exotic coccinelid in chili pepper agroecosystems in Brazil. In the field, aphidophagous arthropods were dominated by Coccinellidae, Syrphidae, Anthocoridae, Neuroptera and Araneae, and these natural enemies were readily observed preying on aphids, feeding on flowers or extrafloral nectaries, and using plant structures for oviposition and/or protection. The most abundant plant species harboring aphidophagous predators were *Ageratum conyzoides* L., *Sonchus oleraceus* L., *Bidens pilosa* L. (Asteraceae), *Digitaria* sp. (Poaceae), *Solanum americanum* Mill. (Solanaceae) and *Senna obtusifolia* (L.) H.S. Irwin & Barneby (Fabaceae). Survival of native *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae) differed between plant species, with significantly greater survival on *A. conyzoides* and *B. pilosa*. However, no evidence was gathered to suggest that weed floral resources provided any nutritional benefit to the exotic *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). It seems likely that native species as *C. sanguinea* have an intrinsic relationship with spontaneous plants and may have developed adaptations to utilize and digest available pollen and nectar. This research has provided evidence that spontaneous weeds in chili pepper agroecosystems can affect aphid natural enemy abundance and survival, highlighting the need for further research to fully characterize the structure and function of plant resources in these and other tropical agroecosystems.

# Enhancing biological control agents of *Helicoverpa zea* and thrips through incorporating flowering resources in corn- cover crop intercropping systems

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Field experiments were conducted to examine effects of intercropping corn with flowering cover crops on: 1) parasitism of corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) eggs by *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae), 2) occurrence of a predator, *Orius* spp. (Hemiptera: Anthocoridae), as influenced by prey *H. zea* eggs and thrips [primarily, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)], and 3) sweet corn, *Zea mays* L. (Poaceae) yield; on Oahu, Hawaii. Sweet corn was grown with three flowering cover crops, buckwheat, *Fagopyrum esculentum* Moench (Polygonaceae), cowpea, *Vigna unguiculata* (L.) Walp., and sunn hemp, Crotolaria juncea L. (Fabaceae) in 2:1 and 4:1 intercropping ratio in 2009 and 2010, respectively. In the year 2010, flowering cowpea and sunn hemp provided an in-field source of *T. pretiosum*, and resulted in higher parasitism of *H. zea* eggs on corn. Buckwheat helped to enhance predator – prey interactions in corn, resulting in reduced abundance of prey in both years. Intercropping in 4:1 ratio negated all possible effects of intra-specific competition, and resulted in similar sweet corn yield in treatment habitats. Incorporating flowering resources in corn may provide sources of natural enemies and can be considered as an important tool for conservation biological control methods.

# Potential biological control agent of the desert locust, *Schistocerca gregaria*: Behavioural characteristics of the predatory beetle *Pimelia senegalensis*

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The desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae), is a serious pest of agricultural crops. After locust control operations involving applications of chemical insecticides in West Africa, environmental assessments are conducted using the richness of animal species and numbers. The darkling beetle, *Pimelia senegalensis* Olivier (Coleoptera: Tenebrionidae), is a representative bioindicator. At the same time, larvae of the beetle reportedly prey on locust egg pods, so the beetle has been considered as a biological control agent to reduce the risk of the harmful effects of chemical insecticides on the environment. Although an understanding of the biology of *P. senegalensis* is essential for the more meaningful biological control and environmental assessments, little information is available on the behavioural characteristics of the beetle in active locust control areas. Therefore, we conducted field observations to examine the behavioural traits of *P. senegalensis* in Mauritania. Pit-fall traps were used to determine diel periodicity of the beetle. Significantly more beetles were found in the traps at night than in the daytime, and sheltering behaviour was observed during the daytime. The beetle was released into an experimental area with different widths and lengths of pipes as shelters in order to verify shelter preferences, and we observed its activity for three consecutive days individually. Significantly more beetles were found inside the smaller and longer pipes, and we demonstrated that they were completely nocturnal to avoid high temperatures in the desert. We also observed whether or not the beetle really feeds on egg pods of the locust, and discussed the efficiency of the beetle as a natural enemy of the locust.

# Evaluation of weed control management on the incidence of the coffee-miner and predatory wasps

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The coffee-miner *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae), is one of the most important arthropod pests in the coffee agroecosystem, usually reaching population levels that require control. Among the leaf miner management tactics, natural biological control is important in regulating populations, in particular predatory wasps that provide predation up to 69% for the soil and climatic conditions of southern Minas Gerais. Thus, in the coffee agroecosystem, special attention should be given to coffee plants, to maintain and increase these natural enemies. The objective of this study was to evaluate the effects of the presence of weeds and weed control in the incidence of leaf miner and predatory wasps. The experiment was conducted in a coffee, *Coffea arabica* L. cv Paraíso (Rubiaceae) in São Sebastião do Paraíso, Minas Gerais. Experimental treatments applied at interrows were: 1) pre-emergence herbicide, 2) post-emergence herbicide, 3) manual weeding, 4) mowing, 5) disk harrowing, 6) rotary tillering, and 7) no weed control. The sides of the coffee rows, about 0.8 m wide were maintained free of weeds by hand weeding. It was observed that the coffee-miner populations are correlated with climatic conditions, increasing in the dry season with warmer temperatures and decreasing with the onset of the rainy season with increased air temperatures. In the first half of 2012 during the prolonged rainy season, low leaf miner infestations occurred in all treatments. However, starting from May at the beginning of the dry season larger infestations were observed in all treatments. Infestation peaks were also observed at the July-September period, returning to low levels in October when the rainy season began, confirming results already found in the southern region of Minas Gerais. When the percent of predated mines was evaluated, the same correlation was observed: the presence of and increasing populations of the predatory wasp was dependant on the growth of the coffee leaf miner populations.

# Conservation of natural enemies a successful example in the field management of African rice gall midge, *Orseolia oryzivora* Harris and Gagné (Diptera: Cecidomyiidae) in Nigeria

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African rice gall midge (AfRGM) *Orseolia oryzivora*, is one of the major insect pests of rice in Africa. Biological control of AfRGM appears to be the most promising control measure using two indigenous parasitoids (*Platygaster diplosisae* and *Aprostocetus procerae*). However, these paarasitoids have not given the desired results despite high levels of parasitism recorded in the field.  This calls for evaluation of the rice ecosystem to assess the biotic linkage with parasitoid build up and AfRGM infestation. Hence, experiments were conducted to determine the influences of conservation of these parasitoids in an alternative host, *Orseolia bonzii* in *Paspalum scrobiculatum*, a common weed in the management of AfRGM.

The conservation strategy adopted in the study was to plant raised seedlings of *P. scrobiculatum* one meter around the border of each rice plot, one month earlier in the season, allowed to be infested naturally by *O. bonzii* and parasitized by these parasitoids, before planting of the rice plants inside each plot. The control experimental plots were planted with only rice seedlings when rice was planted in the other experimental plots, leaving the border empty without any weeds. The host transfer experiments to ensure that these parasitoids can transfer from the alternative host to rice midge were conducted in a screen house.

The results indicated that rice fields with the *P. scrobiculatum* border have very low level of AfRGM infestation due to earlier and higher parasitism with the resultant increase in grain yield compared to the control plots. The results of the host transfer experiment with *Platygaster diplosisae* and *Aprostocetus procerae* from *O. bonzii* indicated that the parasitoids persisted in *O. bonzii* until *O. oryzivora* colonized the first rice crop and then transfers to the rice midge. These results were confirmed by the significant correlation recorded from the study, which justifies its economic usefulness.

The results of the study provided a mechanistic understanding of the important contribution of conservation in the management of AfRGM.

# Session 19 d): Miscellaneous

## Why don't biological agents mitigate the global threat of red palm weevil, *Rhynchophorus ferrugineus*?

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Worldwide, 28 palm species are attacked by the red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), in 46 countries. RPW is native to South and South-East Asia and was discovered in the mid 1980's in the Arabian Gulf from where it moved in early 1990's into Africa (Egypt) and then into Europe (Spain) by the transportation of infested offshoots. In last 3 decades, RPW has been reported world wide from all the continents and prefers to infest young palms 20 years old and less although in the Canary island palm older palms are attacked. The first web site (http://www.redpalmweevil.com) on this globally important pest was established in 1998. A single female can lay about 300 eggs in cracks, crevices and wounds, which then hatch into damage-inflicting grubs. All stages (egg, larva, pupa and adult) are spent inside the palm trunk. Early symptoms are difficult to detect; neither damage nor larva can be seen. Overlapping generations appear inside the palm with serious tissue damage, while a brownish viscous liquid is oozed out and chewed fibers protrude from small holes in the trunk. Infested palms that do not respond to treatments with insecticide have to be eradicated. RPW is currently managed through a pheromone-based Integrated Pest Management (IPM) strategy, where early detection of infestation is the key to success. Implementing international and local plant quarantine regulations is essential. Field sanitation and cultural practices are also important to prevent weevil infestation. There are several reports on the use of biological control agents (mainly nematodes and fungi) that work well in the laboratory or semi field conditions against RPW but do not give the desired results in the field. This is obviously due to the fact that all the stages (egg, larva, pupa and adult) of this pest are concealed inside the palm, making it extremely difficult for the biological agents to access the host. Also, behavior of the pest inside the palm is not very well understood. We therefore need to orient research on biological agents in accordance to the theme of ICE2012 in Daegu, Korea (New Era of Entomology) (http://www.ice2012.org/).

# Structure of tortricid-parasitoid community in blueberry crop of Buenos Aires, Argentina

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The most abundant species of tortricids in blueberries are exophytic leaf and flower feeders (including leaf-rollers); and it is expected that there would be a greater richness of koinobiont parasitoids associated with these tortricids. As a newly introduced crop in Argentina, is probable that food webs in this crop have low complexity. We described the composition of parasitoid guilds and obtained the tortricid-parasitoid food web metrics. Sixty samples were taken monthly during three years in four blueberry fields around Buenos Aires. Parasitoid guilds were recognized following the classification made by Mills (1992). Four species of tortricids and five species of parasitoids were identified. Three parasitoid species were larval koinobiont endoparasitoids, two were pupal idiobiont endoparasitoids, and one was a gregarious ectoparasitoid. Four guilds were present: larval, early larval–prepupa, mid larval ectoparasitoid, pupal endoparasitoid. The early larval–prepupa guild caused the highest percentage of parasitism. The rate of species in the food web showed a trend in favor of the parasitoids. There were 2 species of parasitoids for each species of tortricid. The connectance was 0.086, the density of joints was 0.78, the number of real joints was 7, and the Berger-Parker index was 0.73 for tortricids and 0.59 for parasitoids. The richness of koinobionts was similar to that of idiobionts and most parasitoids were endoparasitoids. The values of the food web metrics suggest that these communities have low complexity and would be susceptible to climatic and anthropogenic perturbations arising from crop management.

# Molecular characterization of parasitoids associated to new described mealybug species in Chile

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Two new mealybug species infesting vineyards and fruit orchards in Chile were described in 2011: *Pseudococcus meridionalis* Prado and *Pseudococcus cribata* González (Hemiptera: Pseudococcidae). The best option to control mealybugs is biological control in view of the several restrictions imposed on some available insecticides, and the lack of efficiency for the remaining ones in the field, mostly due the cryptic behavior of the pest. These newly described species were commonly confused with *Pseudococcus viburni* Signoret, the most abundant mealybug in Chilean vineyards and fruit orchards, which is why no studies are available about their biological control agents. The aim of this work was to survey for the natural enemies attacking these species. Mealybugs collected in fields infested by P*. meridionalis* and *P. cribata* were isolated for parasitoid emergence. Genomic DNA was extracted from the emerged parasitoids using a non-destructive DNA extraction technique. The DNA was amplified by PCR at the markers 28S and ITS2 and the sequences were searched with BLAST, with internal data-bases and international ones such as GenBank. None of the parasitoids obtained had been previously described. From *P. meridionalis* only one parasitoid was emerged, belonging to the genus *Anagyrus* (Hymenoptera: Encyrtidae). In the case of *P. cribata*, a different *Anagyrus* sp. was found. This work corresponds to the first molecular characterization of the parasitoids associated to the new described species of agricultural importance and confirms the presence of natural enemies attacking them. Further work is needed to characterize these parasitoid species morphologically and describe them formally as new species. Funding: CONICYT Doctoral Fellowship #21110864, Conicyt Grant "Tesis de Postgrado en la Industria #7812110011", ECOS-Sud Program-Ambassade de France à Santiago du Chili, EU FP7-IRSES#269196 'Iprabio' and EU FP7-KBBE 'PURE'.

# Natural enemy of powdery mildew in Japan: potentials and problems

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The powdery mildew fungi (PMF) cause powdery mildew (PM), which is a common fungal disease on many kinds of crops around the world. PM is a serious disease and PMF readily develops resistance to fungicides. It is therefore important to establish biological control methods for PM.

Some kinds of insects and mites were known to feeder of PMF. In Japan, four species of the tribe Halyziini (Coleoptera: Coccinellidae) are known to feeder of PMF (Sasji, 1998). The most common species is the yellow spotless ladybug, *Illeis koebelei* Timberlake and it is easily collected throughout Japan. However, there are no reports about the possibility of biological control agents against PM. We investigated whether I. koebelei could be used as a useful tool for the biological control of PM. We investigated 1) life history characters, 2) feeding quantity, 3) flight ability and 4) attractiveness of volatile and contact chemicals to *I. koebelei*.

The optimum temperature for growth was 25°C, the period of adult emergence was 14.6 day, and adult longevity was about 90 days. Maximum PMF consumption per day was 4.9 cm2 in the fourth instar. The estimated average area of PMF consumed was about 24 cm2 per larva, and about 127 cm2 per adult. Females had greater flight ability than males. The peak flight age of females was about 9 days, and this period may relate to mating and oviposition behavior. Methyl salicylate and methyl jasmonate did not attract *I. koebelei*, but an extract of infected leaves attracted adults. An extract of PM-infected leaves may promote the settlement of *I. koebelei*. Also, we investigated problems of using *I. koebelei* for biological control from the point of PMF transmission.

# Complementary biological control strategies: parasitoids, predators and entomopathogenic fungi against the cabbage root fly, *Delia radicum*

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We are investigating the impact of complementary biological control strategies on pest control of *Delia radicum* (L.) (Diptrera: Anthomyiidae), and the interactions between the control organisms. Through niche complementarity of natural enemies in time and space the pest control could potentially be improved. The aim is to combine inoculation biological control, using the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae) and *Metarhizium brunneum* Petch (Clavicipitaceae), with conservation biological control of the specialist parasitoid *Trybliographa rapae* Westwood (Hymenoptera: Figitidae) and the generalist predator/parasitoid *Aleochara bipustulata* (L.) (Coleoptera: Staphylinidae) in the *Brassicaceae* agroecosystem.

Through dual choice bioassays we have evaluated the response of *T. rapae* when encountering fungal infected hosts and fungus inoculated host environments. In dose-response assays, the tested fungal isolates were found to be pathogenic against *T. rapae*, even at low doses. Thus, entomopathogenic fungi pose a potential threat to field populations of *T. rapae*. In dual choice olfactometer trials, *T. rapae* avoided host patches treated with high concentrations of *M. brunneum*. An avoidance was also seen in oviposition assays, where *T. rapae* laid fewer eggs in host larvae treated with *M. brunneum* compared to healthy control larvae. However, *B. bassiana* was not avoided to the same extent by *T. rapae*. Odour collections of cabbage plants inoculated with *D. radicum* eggs and later treated with fungi revealed fungus specific compounds. Electrophysiological antennal recordings of *T. rapae* will be performed with the odour collections.

Furthermore, semi-field cage trials were conducted to evaluate the mortality of *D. radicum* larvae by *T. rapae* and *M. brunneum* individually or in combinations. At low fungal concentrations a complementary effect of both biological control agents was observed. The consequences of introducing fungi to the system will be summarized.

# Assessment of pathogenic fungi of the Red Palm Mite, Raoiella indica, in the Caribbean

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A survey was conducted to isolate and identify pathogenic fungi associated with *Raoiella indica* Hirst (Trombidiformes: Tenuipalpidae) in Trinidad, Antigua, St. Kitts, Nevis and Dominica. In order to isolate potential pathogenic fungi, a total of 920 mites from all four islands were either surface sterilized or left unsterilized before being cultured on tap water agar. A total of 318 fungal colonies were retrieved from the mites. A further 96 mites from Dominica were kept on sterile moist filter paper in a humidity chamber and a further 85 colonies were isolated from the mites. Based on morphological observations of all 403 isolates, a representative sample of 32 (8%) were sent for identification at CABI-UK. However, of these five could not be identified due to bacterial/fungal contamination. Of the 27 fungi positively identified, 15 isolates were of the genera *Cladosporium* (Capnodiales), which include saprophytes, plant pathogens and fungal parasites. Three isolates of *Simplicillium* spp. (Cordycipidaceae), possibly from an unknown taxon, and a *Penicillium* (Trichocomaceae) isolate were considered as having potential for future evaluation for pathogenicity to *R. indica*. Species from other genera identified with limited or no pathogenic potential to control the mite include: *Aspergillus*, *Cochliobolus*, *Fusarium*, *Pestalotiopsis* and *Pithomyces*. The population of mites in Trinidad-Icacos, Trinidad- Manzanilla, Antigua, St. Kitts & Nevis and Dominica was assessed and was found to be 10.69, 5.79, 3.38, 6.17 and 4.94 mites/cm$^2$, respectively.

# Control of diapausing larvae of *Cydia pomonella* in the field using two new species of entomopathogenic nematodes

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*Steinernema feltiae* (Filipjev) strain N22 and *Steinernema austral* Edgington et al. strain N3, (Rhabditida: Steinernematidae), two recently discovered nematode strains, were tested in the field for efficacy in controlling cocooned larvae of the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae). Four doses (100.000 to 400.000 infective juveniles) were sprayed in the winter season once on cardboard bands stapled to apple trees. Larvae were removed 96 h after spraying from cardboard bands and checked. The four doses caused high mortality, ranged from 78-96% to 68-71% for *S. feltiae* and *S. australe*, respectively. *Steinernema feltiae* was more virulent than *S. australe* and was selected for the following experiment. A single dose of 1.000.000 infective juveniles was sprayed to the trunk of apple trees, up to 1,5 m. After 96 h, cocooned larvae were removed from cardboard bands and from the bark by hand. On average, *S. feltiae* caused 64% of mortality vs 9% of mortality in the untreated trees. Larval mortality was higher in the bands (79%) than in the bark (52%). We concluded that both *S. feltiae* and *S. australe* are good potential biological control agents against overwintering *C. pomonella* larvae, a stage not targeted by other techniques of control.

# Apple sawfly, *Hoplocampa testudinea*, control using entomopathogenic fungi

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The apple sawfly *Hoplocampa testudinea* (Klug) (Hymenoptera: Tenthredinidae) is regarded as a major pest in apple orchards of the north temperate zone. It can be controlled by spraying *Quassia amara* L. (*Simaroubaceae*) extract at petal fall. However the use of *Q. amara* is under debate in the EU. Additionally, the short persistence of *Q. amara* and the limited time frame of *H. testudinea* egg hatching make correct timing of the application challenging. Complementary or possibly alternative control measures are investigated as part of a participatory research project comprising growers, advisors and researchers. The effect of inundative soil applications of *Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae) during larval descent to overwintering sites below-ground was assessed. This allows targeting an additional life stage of the pest as well as increasing the time frame for control measures. Persistence of the fungi after application and impact on naturally occurring entomopathogenic fungi in the soil are important factors when a control strategy is designed.

In June 2011, *B. bassiana* was applied to the soil underneath apple trees in an organic orchard at the recommended dose of 12kg/ha. Soil samples were collected immediately after application, and in April and June 2012. Fungal occurrences and densities were evaluated by baiting soil samples with *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) and assessing colony forming units (CFU) on selective agar media.

Densities of *B. bassiana* were higher in treated plots immediately after application while almost no *B. bassiana* was recovered in control plots where *Metarhizium* sp. (Clavicipitaceae) dominated. In April 2012, *B. bassiana* CFUs/g soil were still more abundant than in control plots. However, in treated plots *B. bassiana* was strongly decreased reaching the level of naturally occurring *Metarhizium*. In 2011, the entomopathogenic activity of *Metarhizium* sp. was decreased by the application of *B. bassiana*. According to both soil baiting and CFU evaluation, the level of *Metarhizium* sp. in the soil was restored to background levels in 2012.

Results indicate that inundative application of *B. bassiana* was feasible showing immediate entomopathogenic activity, negligible long-term persistence in the soil and no long-term impact on naturally occurring *Metarhizium* sp.

# Management of white grubs in California blueberries using entomopathogenic nematodes

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The white grub larval stages of *Cyclocephal*a spp. (Coleoptera: Scarabeidae) are recognized throughout much of the world as pests of turfgrass. Until recently in California, white grubs were rarely considered pests outside of turf. However, the introduction of southern highbush blueberry production in California has allowed white grubs to expand their host range and status as a pest. In response to this new problem we began efforts to identify the grub and develop a management plan for it. The grub was identified as *Cyclocephala longula* LeConte. Surveys of beetle stages present in the ground coupled with black-light trap catches documented that *C. longula* are 3rd instar in April then transition to pupae through May, and adults from mid-June to mid-July and produce eggs to hatch by early August. Data showed that adults begin flying about 30 min after dark and can be collected for a period of two to two and half hours. Treatments of *Heterorhabditis bacteriophora* Poinar (Rhabditida: Heterorhabditidae) in the spring and fall were documented in this project to be an effective tool in management programs for C. longula as 1st to 3rd instar larvae. Insecticide treatments in August with imidacloprid were also documented to be effective. Combining all of this information, California blueberry growers have the basic tools to successfully monitor for *C. longula* and should be successfully reduce pest populations to levels below economic damage.

# Acaricidal activities of essential oils against *Oligonychus afrasiaticus*, an important pest of date palm

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*Oligonychus afrasiaticus* (McGregor) (Trombidiformes: Tetranychidae), known as old world date mite, is an important pest that devastates date palm, *Phoenix dactylifera* L. (*Arecaceae*) in the Kingdom of Saudi Arabia. They caused serious damage to fruits in most of the date palm growing areas of the Kingdom. They feed on the immature dates resulting severe fruit scarring. Sometimes, they damage to plants to such an extent that the dates turn brown with scabbed appearance. Such damage has caused subsequent crop losses and rendered the dates unfit for human consumption. Currently, acaricides are the only tool available to Saudi growers to prevent *O. afrasiaticus* damage. Many acaricides are available in the Saudi markets in order to control the mites on date palm trees but their efficacy against *O. afrasiaticus* is questionable. The intensive use of acaricides has led to resistance in many mite species around the globe and their control becomes exceedingly challenging. The current investigation explored for the first time the acaricidal potential of some essential oils for the environmentally safe management of date mites in the laboratory. Our results showed that all the studied essential oils showed acaricidal activities in a dose dependent manner. Only the essential oil extracted from the juice of orange, *Citrus sinensis* (L.) Osbeck (*Rutaceae*) failed to cause 100% mortality even 15-days post exposure. Essential oils from *Zingiber officinale* Roscoe, *Elettaria cardamomum* (L.) Maton, and *Curcuma longa* L. (*Zingiberaceae*), and *Piper nigrum* L. (*Piperaceae*) were found to be highly toxic. However, *Commiphora myrrha* (T. Nees) Engl. (*Burseraceae*) was found to be moderately toxic. Our laboratory results showed that *Z. officinale*, *E. cardamomum*, *P. nigrum* and *C. longa* are promising for the eco-friendly management of date mites, *O. afrasiaticus*, although their field efficacy remains to be evaluated.

# Entomopathogenic nematodes as potential biocontrol agents for major potato pests

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Potato, *Solanum tuberosum* L. (*Solanaceae*) is grown in diverse agroecosystems where it is affected by different insect pests. Farmers main control strategy is applying insecticides. The objectives of our studies were to collect entomopathogenic nematodes in Peru, Ecuador and Bolivia and to evaluate their pathogenicity and efficacy to control major potato pests. Isolates Peru-1, Peru-2, Ecuador-1, Ecuador-2 and Bolivia-1, all of where were different isolates of *Heterorhabditis indica* Poinar, Karunakar & David (Rhabditida: Heterorhabditidae) were highly pathogenic with lethal doses (DL50) of 1.8-77.3 IJ per larva of Andean potato weevils, *Premnotrypes vorax* (Hustache), *P. suturicallus* Kuschel and *P. lathithorax* (Pierce) (Coleoptera: Curculionidae), potato tuber moth, *Tecia solanivora* (Povolny) (Lepidoptera: Gelechiidae), flea beetle, *Epitrix yanazara* Bechyne (Coleoptera: (Coleoptera: Chrysomelidae), scarabs, *Anomala testaceipennis* Blanchard (Coleoptera: Scarabeidae) and leafminer fly, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae). In semi-field and on-farm field experiments in the central highlands of Peru (3400-3800 a.s.l.), the application of isolate Peru-1 reduced tuber damage of *P. suturicallus* by 41.4-81.6% as well as larvae infestation by 53.2-79.6%. In lowland conditions at the central coast of Peru, applications of *H. indica* caused mortality of L. huidobrensis larvae in potato leaves by 55-85% in pot trials and by 39-69% under semi-field conditions. According to these results entomopathogenic nematodes have a good potential as biological agents in integrated pest management of major potato pests.

# Parasitoidism rate and life table parameters of *Aphytis diaspidis* and *Hemiberlesia lataniae*

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Latania scale *Hemiberlesia lataniae* (Signoret) (Hemiptera: Diaspididae) has become a major pest of avocado in Chile and has reached high populations in the fruit because of limited natural control. The objective of this study was to identify the biological parameters of the parasitoid *Aphytis diaspidis* Howard (Hymenoptera: Aphelinidae) as a control agent of latania scale. Postembryonic development, parasitoidism rate, and life table parameters of the parasitoid and scale were determined under laboratory conditions. Postembryonic development and parasitoidism rate varied significantly when *Aphytis diaspidis* was evaluated on distinct latania scale stages, with the highest survival (56%) occurring at the third scale stage, it also had the shortest development time between egg and adult (19.14 d) and the highest parasitoidism rate (66%). The intrinsic growth rate (rm) was higher for *A. diaspidis* (rm = 0.099) than latania scale (rm = 0.068). These laboratory results demonstrate that *A. diaspidis* is an effective parasitoid for decreasing *H. lataniae* populations, especially in the third stage.

# Role of microbial control in strawberry and vegetable pest management in coastal California

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Chemical pesticides are a major part of pest management broccoli, lettuce, and strawberries, which are important commercial crops in coastal California. In general, high product cost, inconsistent field efficacy, and sensitivity to harsh environmental conditions limit the use of biopesticides. However, with mild weather conditions in the California coast where these crops are grown, entomopathogenic fungi such as *Beauveria bassiana* ((Balsamo) Vuillemin) (Cordycipitaceae) are potential tools for incorporating microbial control into IPM. Difficulty in controlling certain pests with current pesticide applications and continued concern for environmental health with some chemical pesticides are other factors that emphasize the need for alternative control strategies. Since several arthropod pests on broccoli, lettuce, and strawberries are susceptible to *B. bassiana*, a few laboratory, greenhouse, and field studies were conducted to explore the potential of this fungal pathogen. In all these studies, *B. bassiana* demonstrated a potential for controlling aphids, Bagrada bug, lygus bug, thrips, and spider mites while appearing to be safer to natural enemies compared to some chemical insecticides. Highlights of these studies will be presented.

# Food plants of host caterpillars affect development of the parasitoid fly *Compsilura concinnata*

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The rice armyworm, *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) is known as a serious pest that infests grass plants including maize, sorghum and rice, but the larvae often feed on a wide variety of plants when their preferred food plants are limited during outbreaks. In this study, we examined the influence of food plants of *M. separata* larvae on development of their tachinid parasitoid, *Compsilura concinnata* (Meigen) (Diptera: Tachinidae). Host larvae were oviposited by a female parasitoid fly only once within 24 hours after they had molted to the final (6th) instar, and the parasitized hosts were supplied with leaves of maize, sorghum, kidney bean or Japanese radish as food plants. The parasitoid survival rate from oviposition to adult emergence was 57.1% in hosts reared on maize, 68.2% on sorghum, 55.0% on kidney bean and 30.0% on Japanese radish. The larval developmental time of C. concinnata was significantly shorter in the maize and the sorghum treatments than in the kidney bean and the Japanese radish treatments. The body size of an emerged adult was significantly larger in the maize and the sorghum treatments than in the other two treatments. Our results indicate that food plants of host larvae affect development of the parasitoid fly in the host body.

# Assessment of entomopathogens for biological control of *Tuta absoluta* (Meyrick) in tomato (*Solanum lycopersicum* L.)

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Tomato, *Solanum lycopersicum* L. (*Solanaceae*) is one of the most important horticultural crops in Colombia, with more than 15,000 ha planted. The leaf miner *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is a key pest of tomato crop, mainly in cool climates. The damage is caused by the larval stage, attacking the crop in early stages, consuming the apex of the plant and thus, affecting the apical meristem. The leaf miner consumes leaves, stems and fruits, thereby causing considerable economic losses. Presently, *T. absoluta* is managed exclusively through chemical pesticides. The inadequate control and indiscriminate use of chemical pesticides is related with a wide variety of environmental, economic and human health problems as well as induced resistance. In the present work, we compared the efficacy of three entomopathogens as biological control options for *T. absoluta*. The treatments and recommended doses were: no control, *Entomophtora virulenta* Hall et Dunn (Entomophthoraceae) (1.0 cc l-1), *Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae) (1.0 cc l-1), *Bacillus thuringiensis* Berliner serovar. *kurstaki* (Bacillaceae) (1.0 cc l-1) and *B. thuringiensis* serovar. *aizawai* (1.0 cc l-1). We developed a bioassay methodology in which the mortality was calculated over time. We used a Completely Randomised Design consisting of five treatments with five replications for each one. The experimental unit consisted of a tomato leaf containing three larvae inside a Petri dish. The treatments were applied to third instar larvae of *T. absoluta*. Mortality was determined daily. The bioassay was carried out inside a climate controlled room at 21±1ºC, 65±10 % R.H. and 12 h L:D. The mortality was determined through the Henderson-Tilton test. The control efficacy for *B. bassiana* and *E. virulenta* were 69.2% and 15.4%, respectively. Both strains of *B. thuringiensis* reached a control efficacy of 100%. The bioassay showed significant differences through the Kruskal-Wallis test (p-value = 0.0003858), with the best control from *B. thuringiensis*. The results of this work confirmed the possibility to include biological agents to control *T. absoluta*, promoting a sustainable and environmentally sound management for this troublesome arthropod.

# Entomopathogenic fungi infecting forest pests in Brazil

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The trend of pest management is focused on the preservation of the environment, with techniques such as the use of biopesticides. Control of microorganisms is a major component of integrated pest management farm, but it is still little used in forest areas, perhaps due to lack of specific isolates. Entomopathogenic fungi bioprospecting could identify species with good pathogenicity and virulence. Actually, it is known that nearly 400 fungi species have the ability to attack pests and mites, which stimulates studies to use these fungi as micoinsecticides. The objective of this work was to identify entomopatoghenic fungi associated with pest species in eucalyptus lands. The study was carried out in planted areas with *Eucalyptus* sp. in the State of Bahia (Brazil). Several parameters tested the presence of fungi, obtained from living and dead insects collected in the field. After harvest and isolation methodology was applied. The isolates were therefore multiplied in SDA, in a B.O.D. incubator at 27 ±2°C and a photophase of 14 hours, during 7–15 days in order to allow a conidiogenesis and confirm the control agent. The fungi were identified using conidia and conidiophores morphology as well as dichotomous keys. Were collected the following forest pests: *Costalimaita ferruginea* (Chrysomelidae); *Thyrinteina arnobia*, *Euselasia apisaon*, *Glena* sp., and *Sarcina violacens* (Lepidoptera); *Psilideos* (Homoptera). The fungi identified by now are *Beauveria bassiana* (Bals.) Vuill., *Nomuraea rileyi* (Farlow) Samson, *Cordyceps* sp., *Verticillium lecanii* (Zimm.) Viégas, *Verticillium* sp. and *Paecilomyces* sp. The huge diversity of entomopathogenic organisms provides evidence of an apparent and endless source of bioinsecticides. The use of fungi will be a promising strategy to control insects-pests in monoculture areas.

# Biology of the African rice gall midge, *Orseolia oryzivora* Harris and Gagné (Diptera: Cecidomyiidae) and parasitoids by *Platygaster diplosisae* Risbec. in Nigeria

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The African rice gall midge (AfRGM) *Orseolia oryzivora*, Harris and Gagné (Diptera: Cecidomyiidae) is one of the major insect pests of rice in Africa and could result in total crop failure in areas where it is endemic. In this study, the biological characteristics of each life stage of African rice gall midge were assessed under laboratory conditions and parasitism by *Platygaster diplosisae* was investigated.

Reproduction in AfRGM was monogenic and the mean developmental period from egg (2.5 ±0.23 days), 1st instar (3.6 ±0.67days), 2nd instar (3.3 ±2.11 days), 3rd instar (3.0 ± 1.13 day), pre-pupa (2.8 ±1.21 days), pupa (6.1 ±0.56 days) to adult totaled 21.3 ±2.74 days. Mean fecundity was 389.4 ±5.12 and longevity was 3.0± 0.02 days for females and 2.5 ±0.23 days for males. Reproduction in *P. diplosisae* was facultatively parthenogenic. Development was polyembryonic (53 ±4.05 progenies per egg), and mean developmental period from egg (13 ±0.11 days), 1st instar (2.2 ± 0.43 days), 2nd instar (0.5 ±0.31 days), 3rd instar (2.5 ±0.17 day), pre-pupa (2.4 ±0.33 days), pupa (6.7 ± 0.20 days) to adult totaled 27.3 ± 1.2 days with mean fecundity (95.2 ±0.21) and longevity of 4.1 ±0.32 days for females and 3.0 ±0.34 days for males. The functional and numerical responses of *P. diplosisae* were host density dependent corresponding to Holling's type III response curves.

# Feasibility of SIT to control *Rhynchophorus ferrugineus* Olivier (Col.: Curculionidae): an integrated physiological, ethological and genetic approach

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Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Olivier, 1790), native to South eastern Asia and Melanesia, is one of the most important pest of several palm species, including cultivated (*Cocus nucifera*, *Phoenix dactilifera*, *Elaeis guineensis*), ornamental (*P. canariensis*) and spontaneous plants (*Chamaerops humilis*). Adult females lay eggs inside young leaves or in wounds of the trunk and larvae bore into palm trees. If infestation is not detected early, infested palms often die. The goal of this work, financed by the IAEA (Vienna, Austria), is to assess the feasibility of the application of Sterile Insect Technique (SIT) in an IPM to control populations of RPW. In this context, our interest has been focused on three aspects: 1) the study of fecundity and fertility of wild individuals, 2) the study of mating system and selection through behavioral bioassays and genetic analyses, and 3) to perform irradiation screening of the weevil, at different physiological stages. For the first objective, adults, mature larvae and cocoons were collected from infested palms in different seasons, while only adults were collected from monitoring traps. Under laboratory conditions, virgin females were able to lay eggs, although the number of eggs produced was significantly lower if compared to mated females. As expected, the fertility of the eggs laid by unmated females was zero. For the second objective, molecular markers (microsatellite loci and SNPs) have been developed. The deep knowledge of the reproductive biology of this species is crucial, in order to correctly plan any SIT program to against this pest. The mating system seems to be highly promiscuous and contrary to the females, males of RPW are extremely active, initiating all sexual interactions. The molecular markers will allow us to evaluate many aspects regarding the mating system and the population structure of the RPW (e.g. sexual selection, inbreeding levels, gene flow and population size estimates, and so forth). The third objctive, related to irradiation bioassays, indicates the range of irradiation doses necessary to sterilize RPW adult males. These data will strongly contribute to clarify the biological cycle of the RPW and will represent the required background to plan future SIT projects to control or eradicate this noxious species in Mediterranean areas.