

**Report for Project USDAAPHIS5179
#58-5436-1-221**

**Assessing Long-term Impact of Leafy Spurge Biological Control
Agents: Conclusions from a 6-year study**

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Abstract

Three thousand black flea beetles (*Aphthona lacertosa*) and 3000 brown flea beetles (*A. nigriscutis*) were released in 1998 at 76 sites in the vicinity of Devil's Tower, Wyoming. At each of these sites, leafy spurge had become the dominant ground cover and had greatly reduced rangeland productivity. These release sites and 33 control sites were monitored for six years after the release to determine how effective the beetles would be in controlling leafy spurge. In the final years of the study, measurements we documented recovery of grass and forbs as leafy spurge cover diminished. We also captured and counted beetles to assess the long-term survival of the beetles. Within three years the beetles had reduced the average leafy spurge cover from 60% to less than 10% on treated sites. Over the next four years we documented steady recovery of the range as average leafy spurge cover continued to fluctuate between 8% and 22%. In general, grass cover increased from 34% to over 80% in the six years following the release.

We found that the brown beetles colonize new areas of leafy spurge more quickly than black beetles; but the black beetles survive in greater numbers at sites where both species have been established for a year or more. Survival of both species of beetles is sufficient for the population to respond to any rebound in leafy spurge cover.

Land managers in the area quickly recognized the effectiveness of the flea beetles as a control agent and, by the 2nd year of the study, they began gathering surplus beetles from the heavily populated release site. Captured beetles were released at other locations where leafy spurge had significantly reduced range quality. By the end of the six-year study period beetles had either migrated or had been transplanted to every spurge-infested site that we monitored, including all 33 of the control sites established as reference for this study.

Introduction and background

The goals of the initial study were 1) to assess impacts of biological control agents (*Aphthona spp.*) released in leafy spurge infestations (as determined both by remote sensing and field investigations), and 2) to document changes in leafy spurge cover and in native grassland communities responding to the control measures. Results of the initial work were published after 3 years of work (Williams and Hunt, 2002). The project was extended to gain insight into survival of beetles and long-term changes in the plant community. The extended study was to: 1) monitor the dispersion and survival of the two flea-beetle species (black flea beetles (*Aphthona lacertosa*) and brown flea beetles (*A. nigriscutis*)), 2) assess the long-term effectiveness of the beetles as control agents, and 3) to document recovery of rangeland by tracking abundance of plant species that occupy sites where leafy spurge cover is diminished.

The initial study was begun in 1997 (ARS Project 58-5436-7-203) under the direction of Drs. David Kazmer and Raymond Hunt. Amy Elizabeth Parker-Williams, a PhD candidate, was the primary researcher for the initial study. Ms. Williams selected, marked, and gathered data for 109 leafy spurge infested sites distributed across a broad region south of Devils Tower, Wyoming (Figure 1). These sites served as the basis of her PhD dissertation; but were revisited in 2003 and 2004 to determine the long-term influence of the flea beetles and to monitor the recovery of plant communities. In 1998 Ms Parker released approximately 6000 flea beetles (*Aphthona spp.*) at 76 of the selected sites. No flea beetles were released at the other 33 sites which were designated control sites. All sites were marked, classified as to

terrain type, and surveyed for general composition of the vegetation community. Leafy spurge was the dominant species at all sites (at many sites it constituted 100% of the vegetation cover when the study was initiated). Control sites were evaluated in a manner identical to the release sites even though no beetles were released at these 33 sites. Williams recorded GPS coordinates and initial conditions at each site and compiled subsequent evaluations of the progress of the biological agents (Williams, 2001; Williams and Hunt, 2002). Her observations and analyses provide the baseline for subsequent monitoring of conditions in the study area. Ms. Williams completed her PhD program in 2001.

Procedure

During the summer of 1998, 3000 black flea beetles (*Aphthona lacertosa*) and 3000 brown flea beetles (*A. nigriscutis*) were released at each of 76 marked sites (Williams, 2001). No beetles were released at 33 control sites, but these sites were subsequently monitored using the same procedures employed at release sites. Estimates of abundance of flea beetles (by species) were recorded for 2000, 2001, 2003 and 2004. Leafy spurge (*Euphorbia esula* L.) canopy cover was estimated, and dimensions of impacted areas were measured in 2000 and 2001. Percent cover of grass, forbs, and woody plant material was tracked to document recovery of the range as leafy spurge cover diminished.

In subsequent summers, these same sites were located using a GPS unit and 1:24,000 U.S.G.S. topographic maps. By the sixth year (summer of 2004), only 104 of the original 109 sites could be found because the markers (numbered fiberglass stakes) were lost or removed. Markers at most sites were damaged, pushed over, or removed (mostly by grazing animals), but most locations were recovered. GPS coordinates typically proved accurate to within fifteen meters of the center stake marking each location.

Flea beetle populations were sampled when air temperature was 70° Fahrenheit or warmer during the 3-4 week period coinciding with peak emergence of flea beetles (late June and early July). At each site, beetles were captured along six transects using a sweep net and a standardized procedure. Transects were swept over a distance of 20 meters extending outward from the center (beetle release point). One sweep was made per pace (Figure 2).



Figure 2. Melanie Williams capturing beetles with a sweep net, July 2004.

Typically, 5 sweeps along each transect proved sufficient to capture a representative number of beetles. Netted beetles were counted (black & brown) and the numbers were recorded. At sparsely populated sites it was necessary to make 10 sweeps on each transect to obtain a representative sample of beetles. At these sites, the resulting beetle numbers were normalized

(divided by 2) to obtain comparable values. The 3-meter offset from center was introduced to avoid disturbance and “over-sampling” at the release point. If the edge of the leafy spurge patch was encountered at a distance less than 23 meters from the release site, the actual number of sweeps was recorded along with the number of beetles so that the values could be normalized for comparison.

The pattern below illustrates the layout of transects (Figure 3). At each site a wire template was positioned over the location stake to indicate ten radial transect directions. The wire pointers are 30 degrees apart and mark five up slope and five down slope transects. The sample design was originally implemented to allow determination of preferred directions of migration of the flea beetles relative to the slope (Williams, 2001). The six transects sampled for beetle numbers (three up slope and three down slope) were randomly selected from the ten possible directions. The other four transects were evaluated for vegetation cover at distances of 2.5 and 7 meters outward from the release point. The slope-parallel direction was used for reference only and was not sampled.

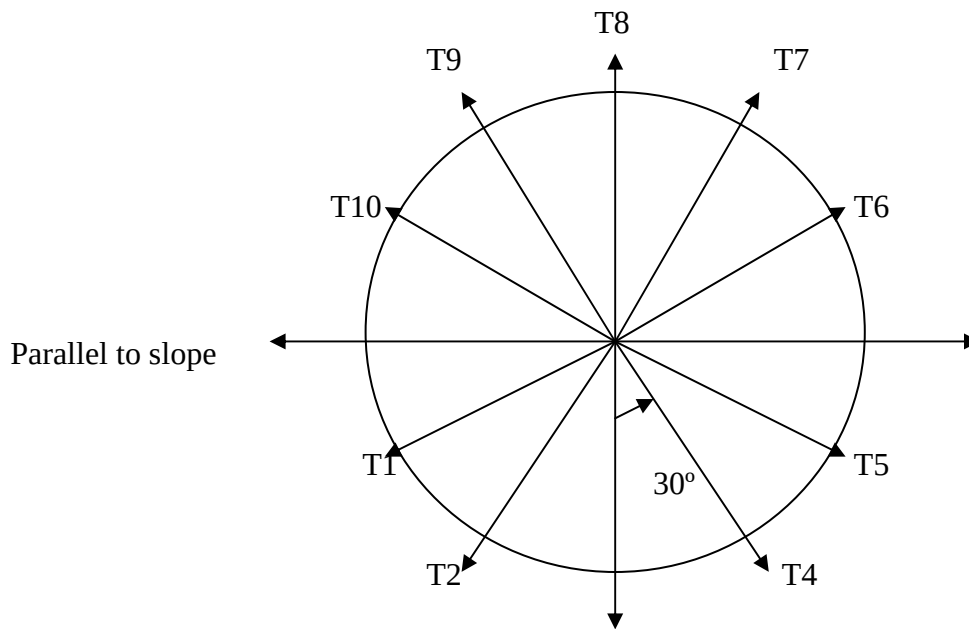


Figure 3. Illustration of the wire template used to identify transect directions.

Composition of vegetative canopy was estimated with the aid of Daubenmire frame (Figure 4). The Daubenmire frame measures 20 cm by 50 cm. Along each of two transects, three frames were dropped approximately 2.5 meters from the center stake. Three frames were dropped 7 meters from the center stake on the remaining two transects. Plants within each frame were identified and percent cover was estimated. Thus, a total of 12 frame areas were used to estimate the average vegetative canopy cover at each site (Daubenmire, 1959). Estimates of percent cover were made to the nearest 5%, (i.e. 5%, 10%, 15%, etc.). The aspect (azimuth the down slope direction) at each site was also recorded. For general analyses, the different cover types were grouped into broad categories as defined by Williams (2001): grasses, forbs, woody plants, bare ground and leafy spurge.



Figure 4. Melanie Williams and Rachel Shorma identifying plants and estimating vegetative canopy cover using the Daubenmire frame, June 2004.

Five photos were taken at each site during each summer campaign (upslope, down slope, left of upslope, right of upslope, and one photo of the area surrounding the stake). These were used for visual comparison of change in plant cover and condition over time. In each case we photographed the release or control site from approximately the same position. Up slope and down slope photos recorded context and changes across the broader area. Figure 5 is a typical comparison the time when beetles were introduced in 1998 (left) and after the beetles had occupied the site for six years and other vegetation types had been allowed to recover (right).



Figure 5. Comparisons of photos taken at site #19 in 1998 (left) and 2004 (right)

Dispersion and Survival of Flea Beetles

The first two years of the program demonstrated that flea beetles can have a dramatic effect on dense stands of leafy spurge with overall reduction of leafy spurge cover from 49% to 6% on treated sites. These early results prompted ranchers and Crook County weed & pest workers to begin gathering beetles from the release sites for distribution at other spurge-infested sites (such activities began in summer, 2000). The locations of these “collections” and “releases” were not recorded even though the release sites established for this study were primary targets for collection of beetles. Unrecorded release sites were also within the study area and some were near designated control sites. By 2003, full scale beetle “round ups” were underway and captured beetles were being redistributed to other sites in the study area (and over a considerably broader region). Consequently, transfer of this new technology to local land managers occurred with no input from project scientists. It also became apparent that statistical analyses of the beetle counts (after summer of 2001) would be distorted by local efforts to establish the beetles across a broader area.



Figure 6. Black and brown flea beetles clustered on the stem of a leafy spurge plant.

By summer of the 2004, black flea beetles had populated almost every release site in significantly higher numbers than brown flea beetles (Figure 7). Williams' early results (2001) revealed a clear tendency for the black beetles to survive in greater numbers than brown beetles (in 2001 the ratio generally was 2 to 1). Subsequent counts show that this trend continued and the disparity has grown larger. After six years, black flea beetles are 3 times more abundant than brown flea beetles on release sites. Cawthra (2001) examined low male/female sex ratios of brown flea beetles as a possible influence on survival rates, but found that sex ratios were not the cause of lower survival rates. With both black and brown beetles, we observed a large increase in beetle numbers for the first 2-3 years, then decreasing populations for three years followed by another increase in numbers of beetles (Figure 7). We believe this pattern indicates that the beetle populations increase and diminish in response to changing abundance of leafy spurge.

It appears that beetle numbers increase until leafy spurge cover diminishes then decline in direct response to reduced food supply. The beetle population response lags behind changes in leafy spurge cover by about 2 years.

A marked increase in the beetle population (in 2004) corresponds to a rebound in leafy spurge cover (in 2002 at release sites and in 2003 at control sites) that occurred when fewer beetles were present. It is possible that the cyclic pattern may moderate as leafy spurge and the beetle populations approach equilibrium. The chart showing increasing numbers of beetles at control sites (Figure 8) suggests that the brown beetles tend to move into new areas more readily than the black beetles. However, black beetles soon follow and their population continues to increase until they become more abundant than brown beetles.

Our counts show brown beetles present in greatest numbers and in larger percentages in the moist, riparian areas. This observation runs counter to previously reported trends in which brown beetles were more abundant in open, dry habitats (Gassmann et al., 1996). The more dense populations of both brown and black beetles in riparian areas is likely due to the healthier stands and more rapid recovery of leafy spurge.

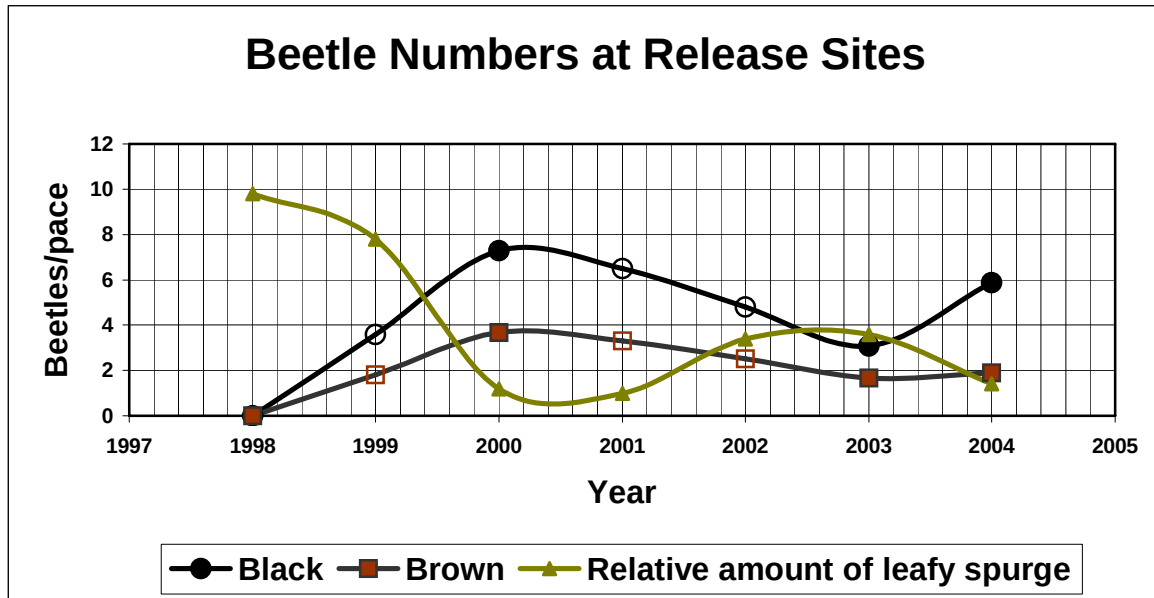


Figure 7. Comparing changes in % leafy spurge cover and number of black and brown beetles at release sites. Leafy spurge declines sharply as beetle numbers increase. When beetle numbers decline, leafy spurge begins to recover for a time. (Note: Numbers shown for 1999, 2001 and 2002 are interpolated).

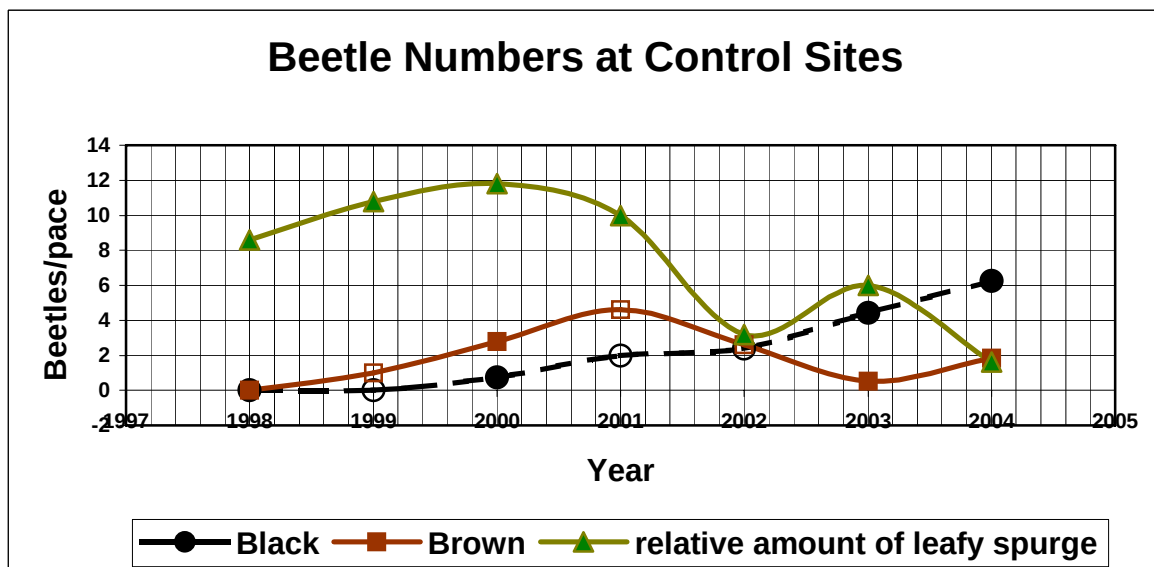


Figure 8. Changes in % leafy spurge and in the number of black and brown beetles at control sites during the 6-year study. Brown beetle populations increase rapidly and then decline. Black beetle populations increased steadily. (Note: Values for 1999, 2001 and 2002 are interpolated).

Both black and brown beetle populations increase in proportion to the availability of leafy spurge (Figures 7 and 8). In areas where leafy spurge cover drops to 10% or less, the population of flea beetles is very low and may represent a maintenance population that will increase as leafy spurge recovers from the initial onslaught of the beetles.

In general, leafy spurge cover is highest in riparian areas (Figures 9 and 10). This is due (in part) to better growing conditions, but it may also reflect the influence of continual reseeding of leafy spurge along watercourses. Leafy spurge seed can germinate in water, thus putting river basins at greater risk for new infestations (Selleck, 1959). Sites 67 through 84 lie in the riparian zone along the Belle Fourche River. Measurements from these 18 sites were compared. The results demonstrate that the leafy spurge cover and beetle numbers decline progressively with distance from water (Figure 9). Beetle density was particularly high within 125 meters of the river. Little change in beetle number is evident beyond 125 meters from the river.

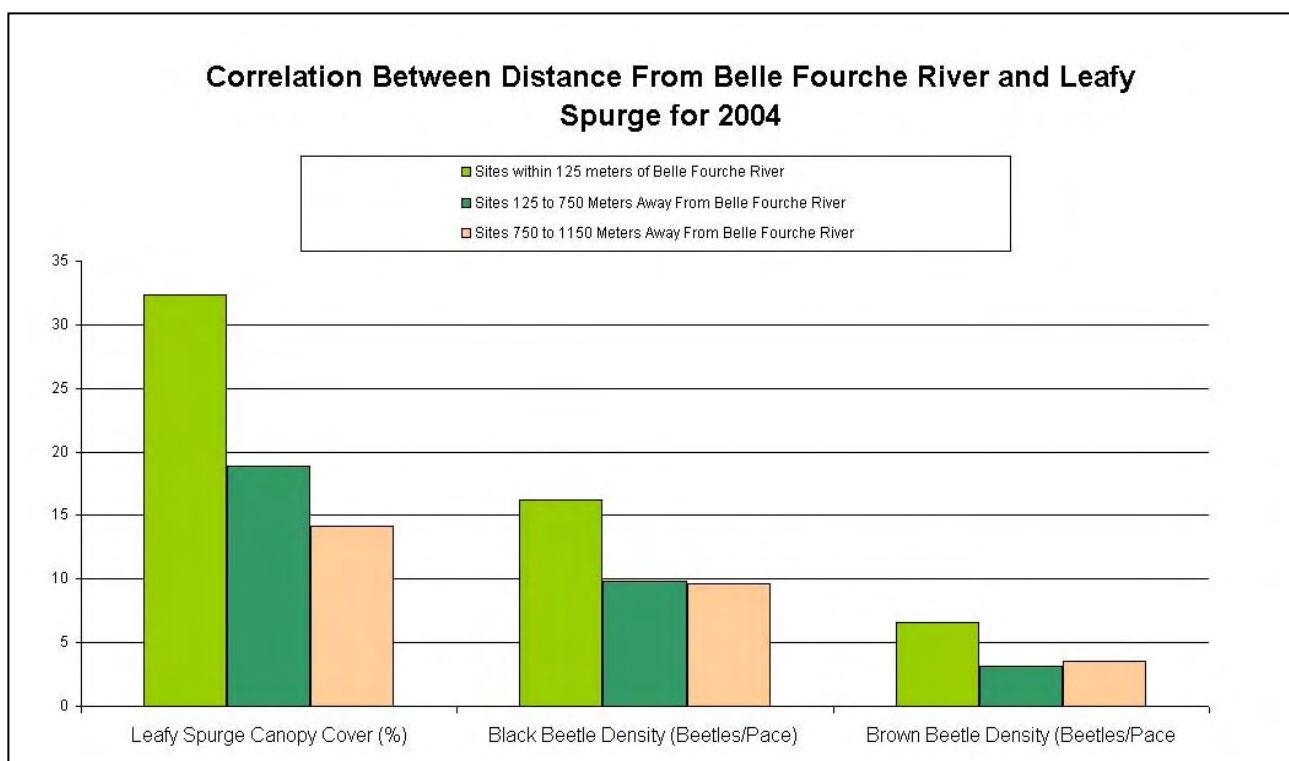


Figure 9. Canopy cover of leafy spurge and beetle numbers decreases progressively with distance from the Belle Fourche River. These data summarize measurements from sites 67-84.

After six years, flea beetles survived in greatest abundance in the riparian areas where leafy spurge cover is highest. Beetle counts in draws netted slightly fewer beetles than in upland areas (figures 10a and 10b). We found no consistent variation in beetle populations relative to slope aspect (Figures 10c and 10d).

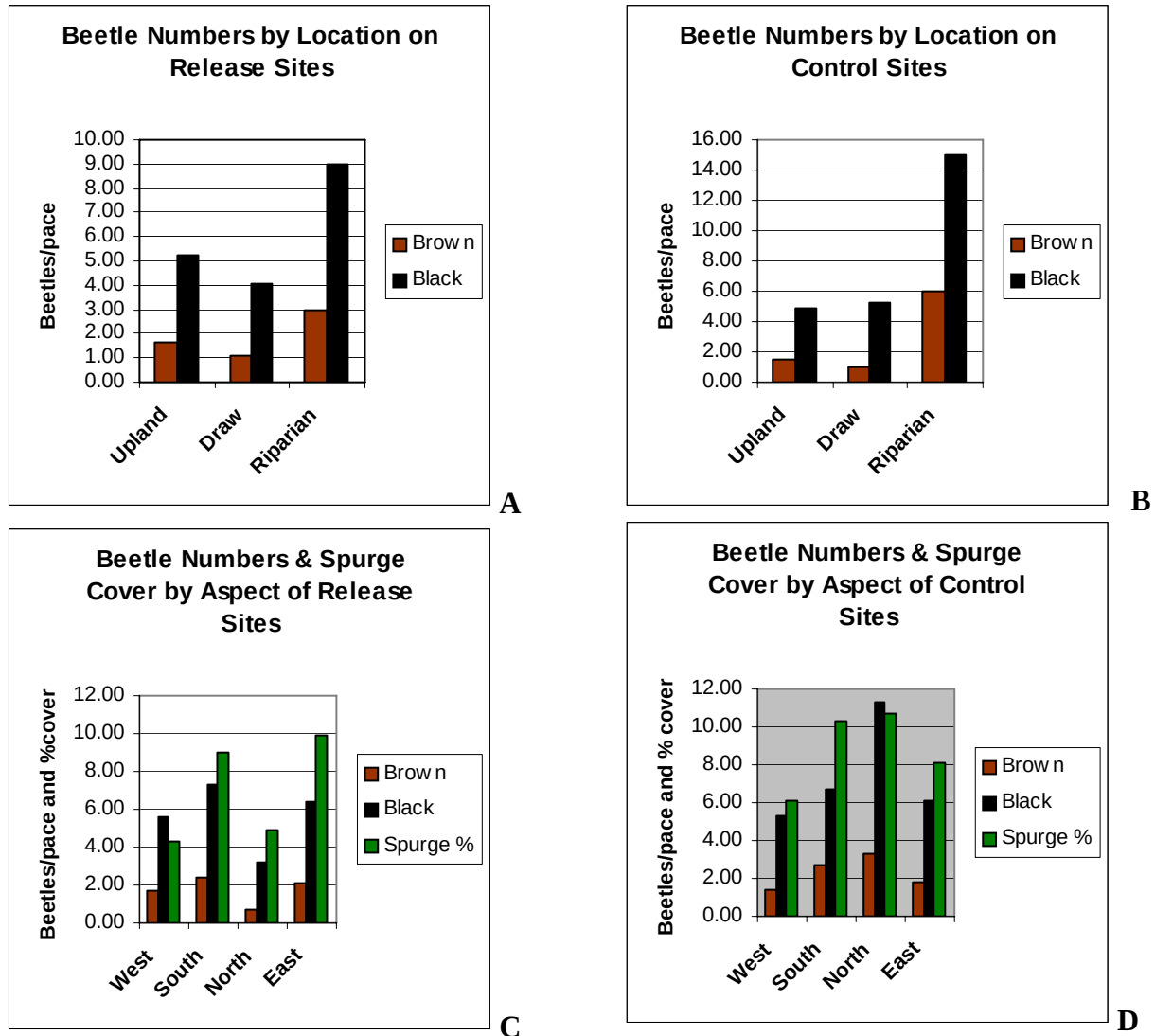


Figure 10. Comparison of numbers of beetles on release and control sites. Top charts (A & B) show differences relative to topographic location and lower charts (C&D) show differences relative to slope aspect. Note: The beetle counts displayed here are from the 2004 field campaign.

Possible Influence of Climate Variation from Normal

The study area was subject to draught during the last four years of the study (Figure 11). The shortage of spring/summer rain and total annual precipitation was particularly acute in 2001. The unusually dry conditions undoubtedly diminished leafy spurge cover and would also have slowed the recovery of rangeland. We see that leafy spurge cover was at a minimum on beetle release sites in 2000 and 2001 (before the onset of draught). This suggests that flea beetles (rather than dry conditions) were the primary influence in diminishing leafy spurge cover. In contrast, control sites show minimum leafy spurge cover in 2002. This decrease in cover may have been caused in part by the severe draught that began in 2001, but it may also be due to the influence of beetles that arrived on the control sites in 1999, 2000, and 2001. The draught may also have affected beetle populations. Beetle populations declined on release sites in 2002 and 2003, but began recovering in 2004. Brown beetle numbers also declined on control sites in 2002 and 2003, but numbers of black beetles increased steadily on control sites. Overall, the changes in beetle populations appear to be linked directly to changes in leafy spurge cover; but they may also exhibit a secondary response to draught.

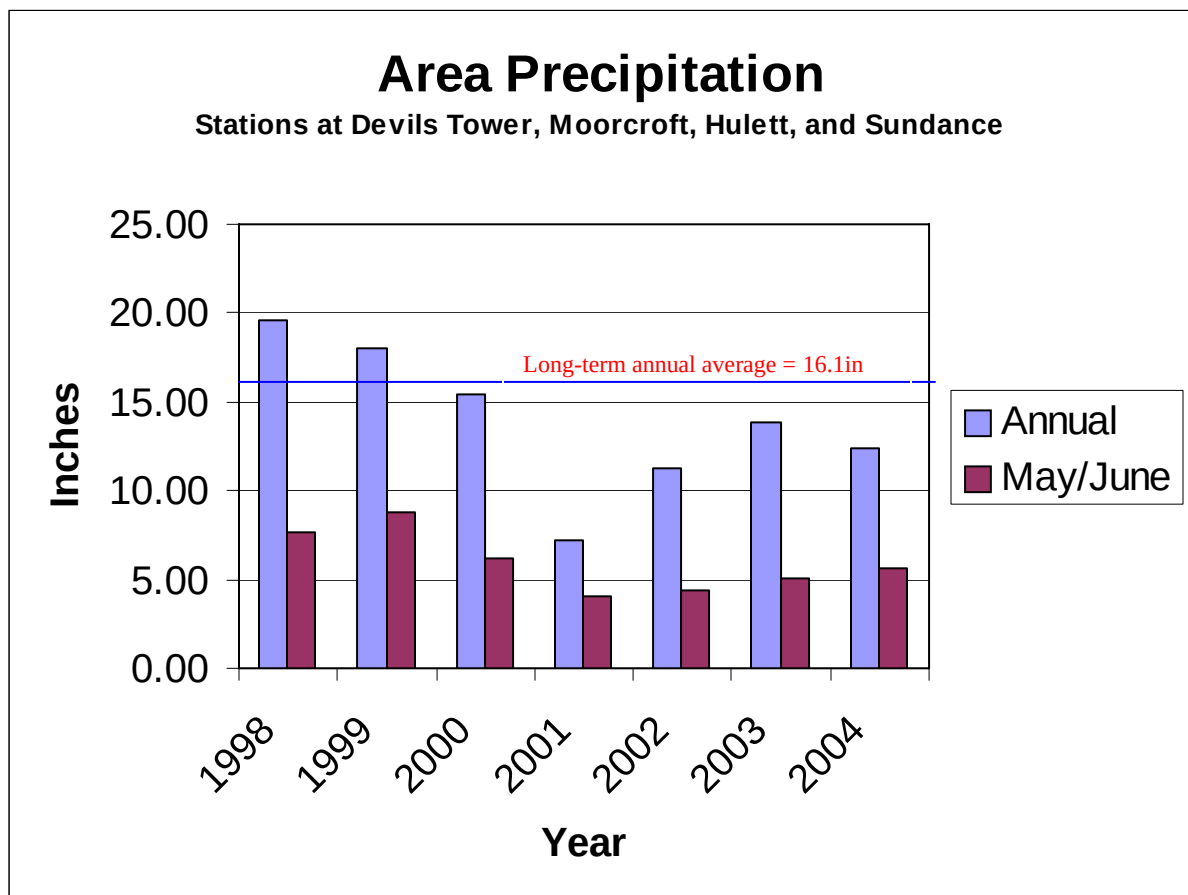


Figure 11. Graph showing annual and early summer precipitation during the study (1998-2004). Draught began in 2001 and has continued through 2004, although it was less severe in 2003 and 2004.

Recovery of Rangeland and Long-Term Effects on the Plant Community

During the initial 2-year period following release of the beetles (1998-2000), leafy spurge declined sharply (from an average cover of 49% to only 6%) in the release areas (Williams, 2001)). The average area of impact grew to 285 m² in the first 2 years. The impact areas spread outward more rapidly on sandy (dry) sites; but the greatest reduction in percent leafy spurge (change in canopy cover) occurred on treated riparian sites. Control sites (untreated) showed an average increase in leafy spurge canopy cover (from 43% to 59%) during this same 2-year period. By 2003, many of the impact areas had merged or reached the boundaries of the leafy spurge patches; so, measurement of impact areas was discontinued.

Charts shown in figures 12 illustrate the recovery of rangeland on release sites and figure 13 depicts vegetation changes occurring on control sites during the same period. Note that the values plotted are the relative abundances (normalized to 100%) for the four broad cover types (rather than estimates of percent cover). From 1998 through 2004, the proportion of leafy spurge decreased from 61% to 9% on release sites, although we observed a brief rebound in the proportion of leafy spurge (up to 22%) in 2003. At the same time, grass increased from 34% to 81% and forbs increased from 2% to 6%. It appears that forbs cover increases initially as leafy spurge cover is reduced; but forbs then decline as the grass continues to take over.

Similar changes were observed on control sites, but the effect of the flea beetles was not apparent on control sites for 2 years after the initial release. During this period the beetles gradually spread from the heavily populated areas surrounding release sites to the under-populated control sites. By the end of the 6-year period represented in this study, the relative abundance of leafy spurge decreased to 10% on control sites, while grass increased to 82%. Change was most rapid between summer 2003 and summer 2004. The very striking inverse relationship between the relative abundance of grass and leafy spurge is shown in Figure 14.

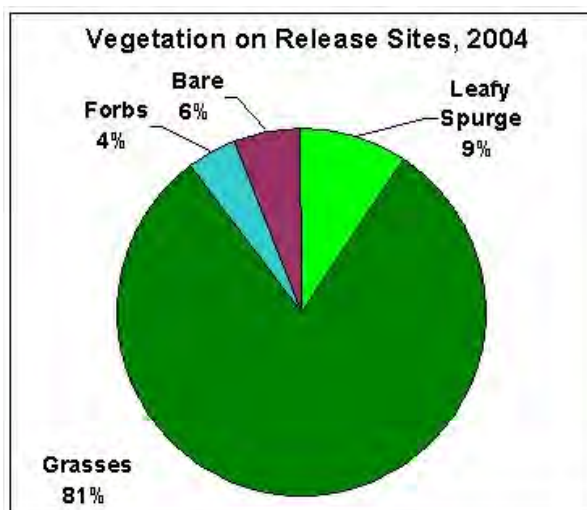
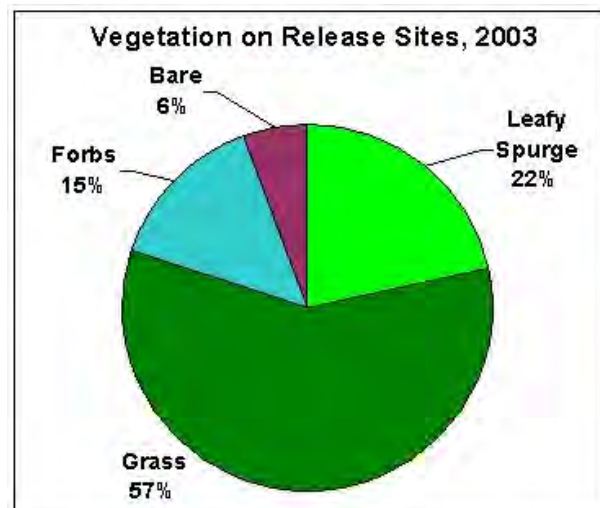
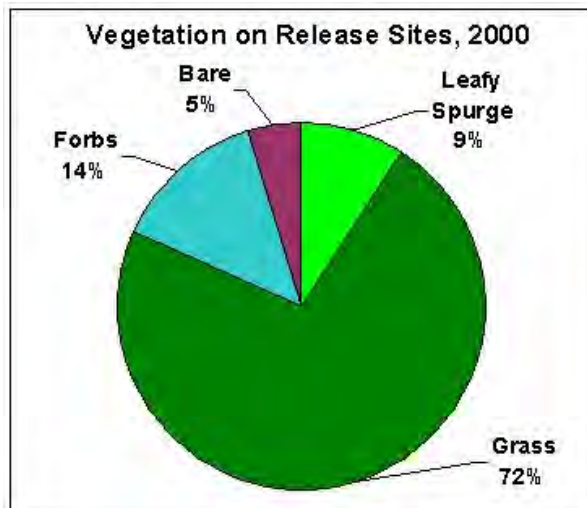
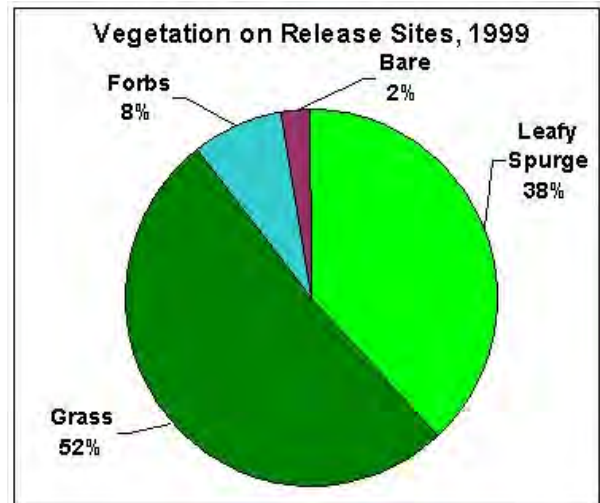
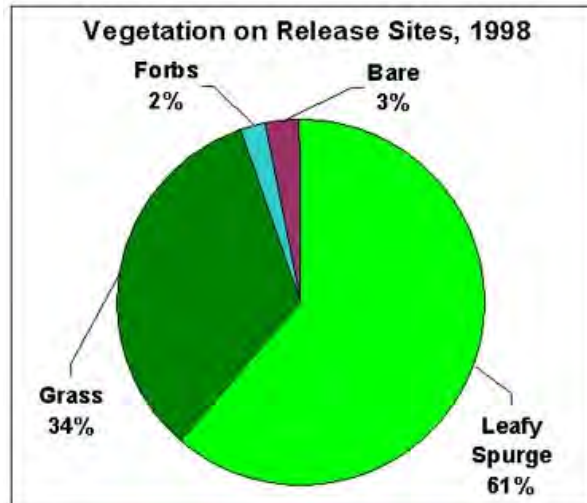


Figure 12. Changes in vegetation cover on release sites for 1998 through 2004. Permanent vegetation types, such as trees and sagebrush, were left out of the comparison because they show little change. The four main cover types that are strongly affected by the changing abundance of leafy spurge are compared. In each case, the four classes presented have been normalized to 100%. The onset of drought in the area began in 2001 and continued through 2004. However, spring and summer precipitation was least in 2001 and 2002 (Figure 11). The drought undoubtedly influenced the recovery rate of grass and forbs.

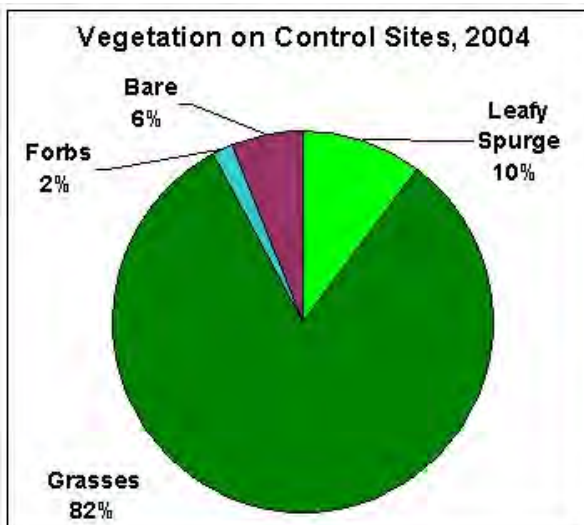
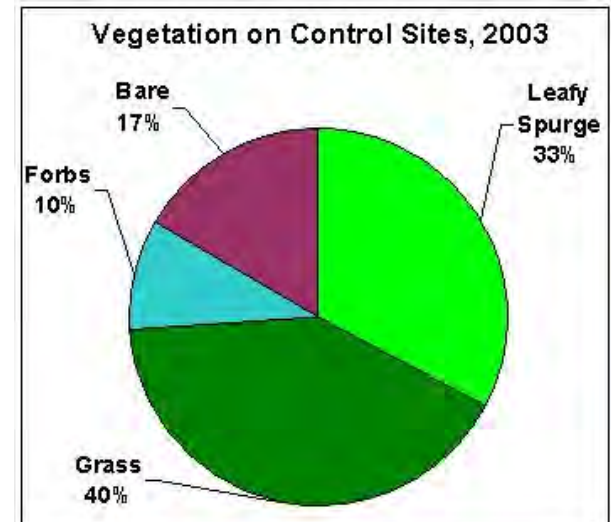
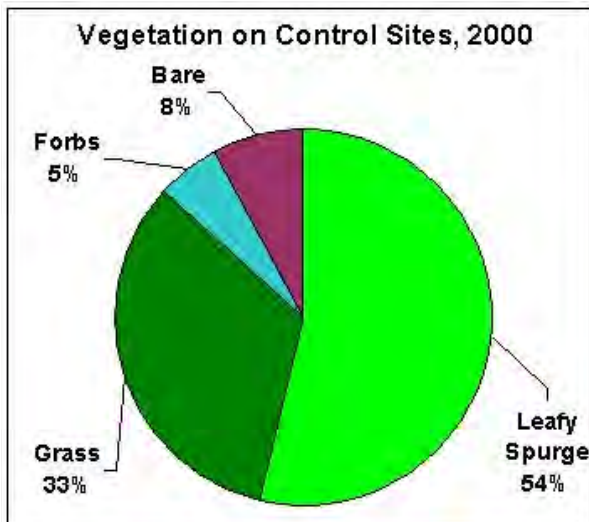
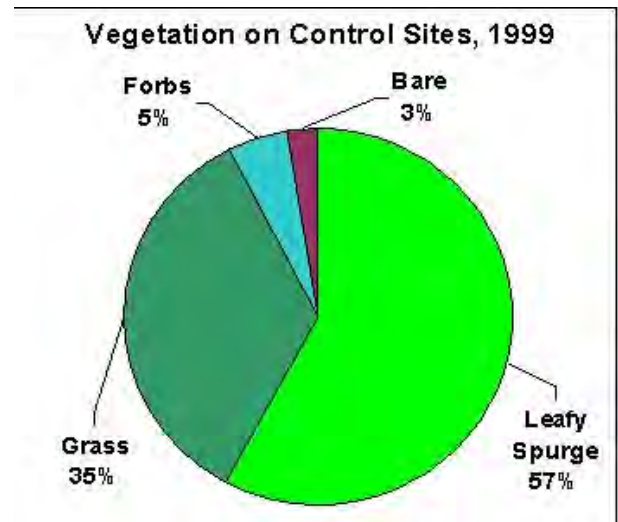
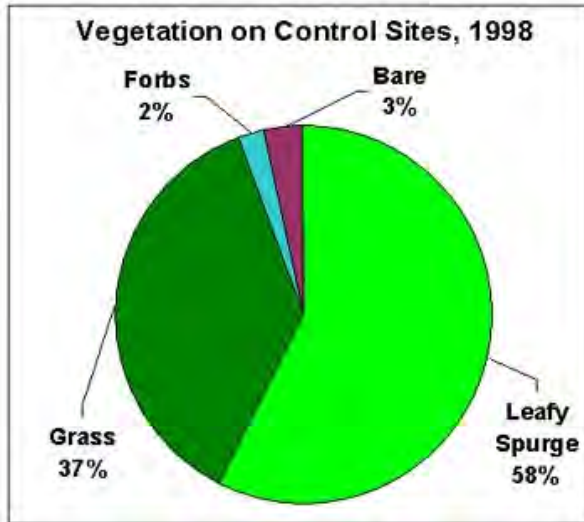


Figure 13. Average changes in vegetation cover on control sites for 1998 through 2004. Permanent vegetation types, such as sagebrush and trees) were left out of this comparison because they show little variation. Instead, the four main cover groups that are strongly affected by changing abundance of leafy spurge are compared. The cover percentages for the four classes presented have been normalized to 100%. Drought began in the area in 2000 and continued through 2004. However, spring and summer precipitation was least in 2000 and 2001 (Figure 11). The drought undoubtedly influenced the recovery rate of grass and forbs.

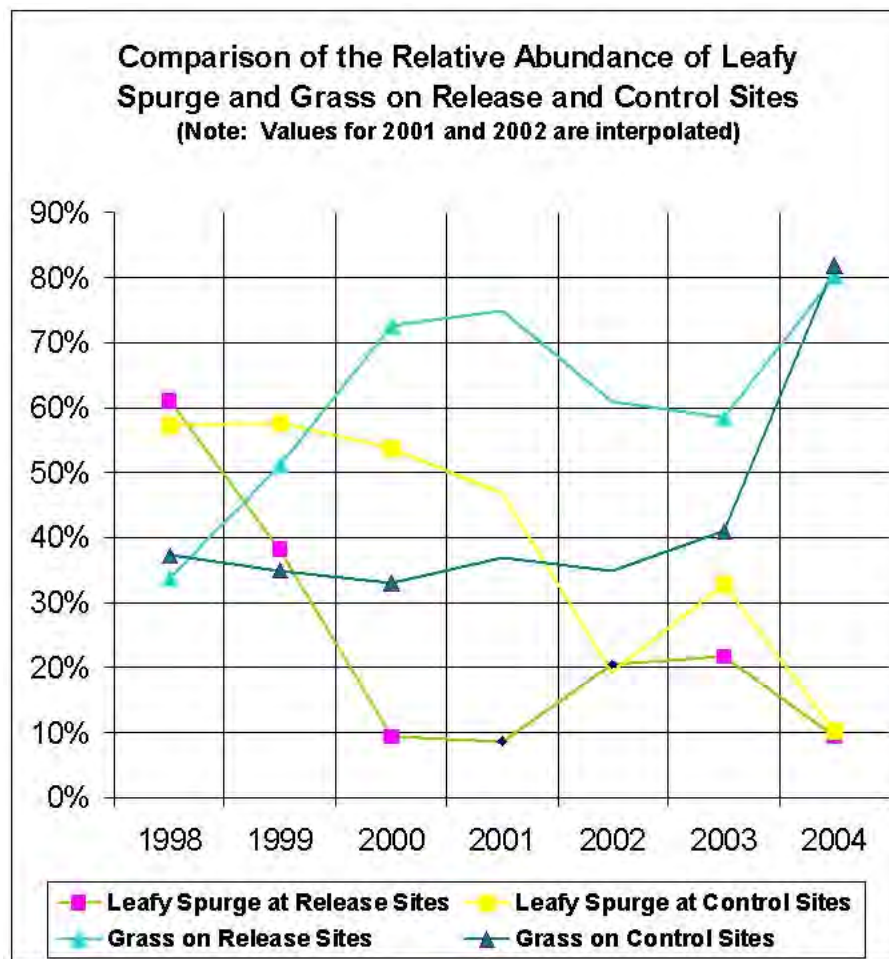


Figure 14. This graph shows the strongly inverse relationship between the relative abundance of leafy spurge and grass.

Comparing general types of sites (upland, woodland, draw, riparian) we see that spurge is particularly persistent in riparian areas and is sparse in draws and on upland areas that have been impacted by flea beetles. Woodland areas are intermediate both in the amount of remaining leafy spurge and in the increase in the abundance of grass.

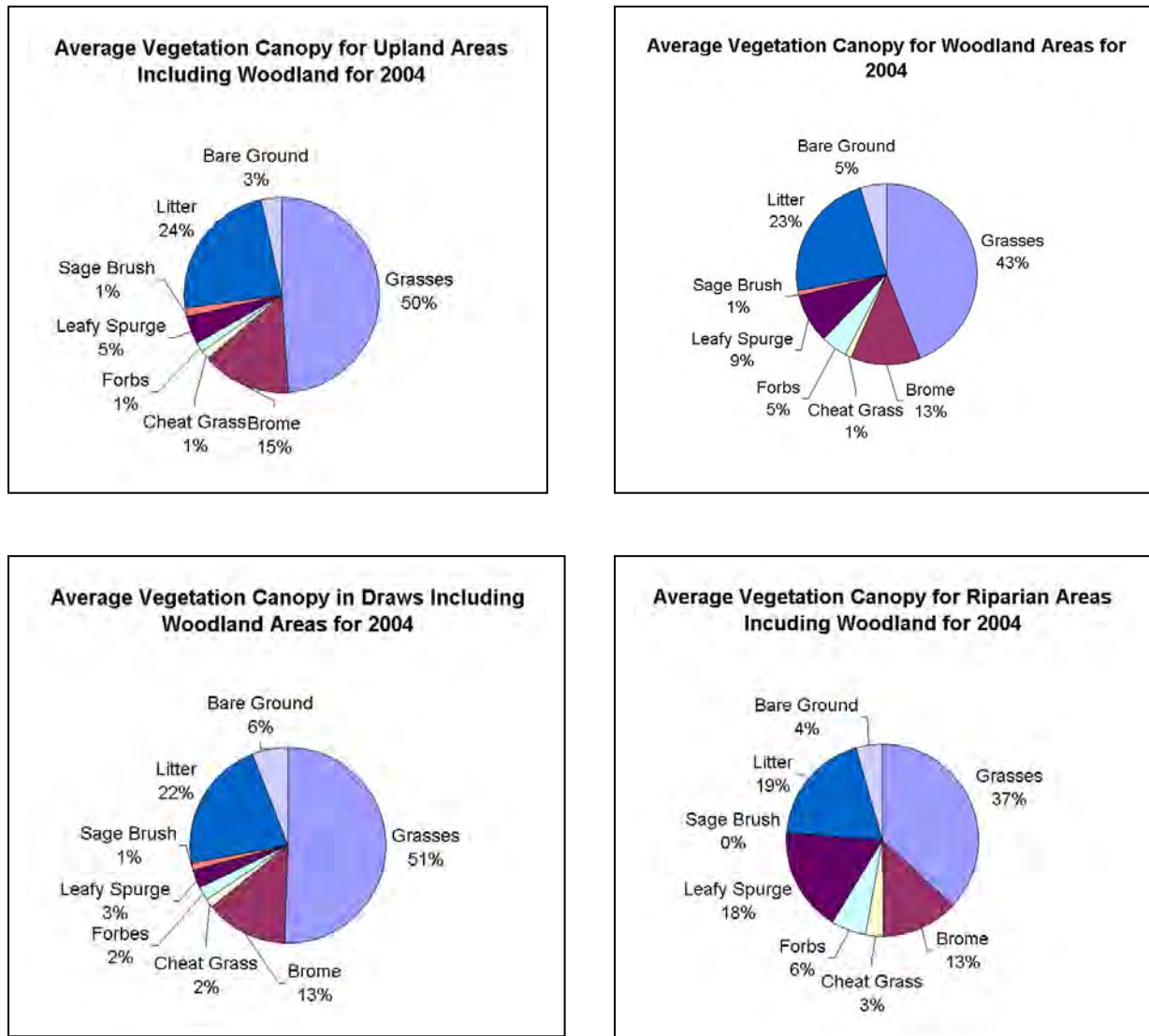


Figure 15. Estimated percentages of different types of vegetation cover on each of the four different site types in 2004. Both control and release sites are included in each diagram.

Conclusions

- Neither black nor the brown beetles showed a significant tendency to migrate more rapidly up or down slope (Williams and Hunt, 2002).
- In this area, the black beetles (*Aphthona lacertosa*) survive in greater numbers than brown flea beetles (*A. nigriscutis*).
- Leafy spurge cover was markedly diminished on beetle release sites within three years.
- By the end of the 3rd year (2001), beetles had colonized many of the control sites and spurge cover declined sharply in the 3rd and 4th year (2002 and 2003).
- Brown beetles disperse more quickly into new areas, but the population of black beetles eventually overtakes the brown beetle population.
- After the initial increase in population (following successful introduction of the beetles in a spurge-infested area), beetle numbers fluctuate in response to the availability of leafy spurge. The beetle population cycles tend to lag changes in leafy spurge cover by one or two years (the lag time is difficult to determine due to the secondary affects of short-term climate fluctuations).
- Six years after the initial release of beetles, spurge cover and beetle populations are highest in riparian areas where moisture and seed transport favor the spread and growth of leafy spurge.
- Flea beetles show no strong preference for any particular slope aspect.
- The onset of draught in 2001 hindered recovery of rangeland vegetation. It may also have had a direct affect on leafy spurge cover and on the beetle populations.
- Grass colonizes areas where spurge is diminished and its abundance increases directly leafy spurge diminishes.
- Forbs are considerably less abundant than grass in recovered areas. Increase in forbs peaks at with the initial reduction in spurge cover, but recedes again as grass becomes dominant.

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Appendix A

Pairs of photographs illustrating recovery of rangeland across the study area: leafy spurge cover was controlled by flea beetles (*Aphthona lacertosa* & *A. nigriscutis*)

Note: Examples are presented for sites where beetles were released in 1998 and for control sites where no beetles were released.

Appendix A, Section 1: Examples of sites in draws where flea beetles were released.



Figure A1: Photographs taken at release site #20. The top photograph (1998) shows a broad draw dominated by leafy spurge. The entire area has fully recovered by 2004 (bottom). The white stake visible in the upper photograph (arrow) marks the release site.



Figure A2: Photographs taken at release site #22. The left photograph shows a draw and slope in the distance, both dominated by leafy spurge in 1998. The entire area shown in this view has substantially recovered by 2004 (right). The white stake at the release site is just visible in the 1998 photo (arrow).



Figure A3: Photographs taken at release site #23. The left photograph (1998) shows a broad draw and hill slope covered by leafy spurge. The site has almost fully recovered and the dead tree has fallen by 2004 (right). The white stake at the release site is visible in the 1998 photograph (arrow).



Figure A4: Photographs taken at release site #34. The 1998 (top) photograph shows a dense stand of waist-high leafy spurge in a broad draw. Relatively few leafy spurge plants remain in 2004 (bottom). Note that the white stake at the release site is seen in the upper photograph near the yellow arrow.



Figure A5: Photographs taken at release site #105. The 1998 (left) photograph shows a draw and slope covered by leafy spurge. Very few leafy spurge plants remain in 2004 (right). This area was a control site. The eradication of leafy spurge was accomplished by flea beetles that moved into the area from neighboring areas.



Figure A6: Photographs taken at release site #106. The 1998 (left) photograph shows a dense stand of leafy spurge in a wooded draw. Few leafy spurge plants remain in 2004 (right). The white stake at the center location of this control site is indicated by the yellow arrow. No beetles were released here in 1998. The change in leafy spurge cover was affected by beetles migrating into the area in subsequent years.

Appendix A, Section 3: Changes in leafy spurge cover on upland release sites.



Figure A7: Photographs taken at release site #40. These photos compare an upland area of leafy spurge as it appeared in 1998 (top) and in 2004. The yellow area indicates the stake at the release site.



Figure A8: Photographs taken at release site #54. These photos compare a wooded upland release site as it appeared in 1998 (left) and in 2004 (right). The arrow indicates the release site.



Figure A9: Photographs taken at release site #55. An open upland release site is covered by leafy spurge in 1998 (top). Almost no leafy spurge is seen in the 2004 photo. The yellow arrow points to the beetle release site.



Figure A10: Photographs taken at release site #59. These photos compare a broad, open upland area as it appeared in 1998 (top) and in 2004 (bottom). The grassland has fully recovered.

Appendix A, Section 4: Changes in leafy spurge cover on control sites in upland areas.



Figure A11: Photographs taken at control site #51. The upper photograph shows leafy spurge covering a large upland region in 1998. In the lower photo we see grass dominating the rangeland in 2004. Beetles were released in this area in 1998.



Figure A12: Photographs taken at control site #100. The top photograph shows leafy spurge in a wooded upland as it appeared in 1998. In the 2004 photo (bottom) we see the recovering grass with sagebrush. No beetles were released at this site in 1998.

Appendix A, Section 4: Changes in leafy spurge cover on release sites in riparian areas.



Figure A13: Photographs taken at release site #68. The 1998 (top) photograph shows a riparian area covered by leafy spurge. By 2004, we see only grass and sagebrush with a few stalks of leafy spurge. The release site (white stake) is indicated by the yellow arrow.



Figure A14: Photographs taken at release site #75. The 1998 (top) photograph shows a broad, wooded riparian area covered by leafy spurge. By 2004, we see that grass dominates the vegetation. The release site (white stake) is indicated by the yellow arrow.



Figure A15: Photographs taken at release site #82. The 1998 (top) photograph shows a sloping riparian area covered by a dense stand of leafy spurge. By 2004, we see that leafy spurge is greatly reduced. The release site is indicated by the arrow.



Figure A16: Photographs taken at release site #94. The 1998 (top) photograph shows a narrow, sloping riparian zone covered leafy spurge. The 2004 image (bottom) shows only grass and a few leafy spurge plants.

Appendix A, Section 6: Changes in leafy spurge cover on riparian control sites.



Figure A17: Photographs taken at control site #67. The left photograph shows an area along the Belle Fourche river covered by leafy spurge in 1998. The right photo shows approximately the same area in 2004. The white stake is at the center of the site (arrow). No beetles were placed at this location.



Figure A18: Photographs taken at control site #81. The riparian are covered by leafy spurge in 1998 photo (left) shows only a light stand of leafy spurge in 2004 (right photo). The white stake marks the site (arrow). Beetles were not released here.