Efficacy of Flea Beetle Control of Leafy Spurge in Montana and South Dakota

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Abstract

Black (*Aphthona lacertosa* and *Aphthona czwalinae*) and brown (*Aphthona nigriscutis*) flea beetles are among the more successful biological control agents used in the control and management of leafy spurge on a relatively large scale in the Northern Great Plains. The objectives of this study were to document leafy spurge population dynamics in response to control by black and brown flea beetles, determine the role of selected site characteristics on establishment and persistence of the beetles, and evaluate the general response of the resident vegetation to control of leafy spurge. In late June 1998, about 3 000 insects of each species were released into permanently marked plots in northwestern South Dakota and southeastern Montana. Beetle abundance, density and foliar cover of leafy spurge, and foliar cover of the resident vegetation were evaluated each year from 1998 through 2004. Black beetles increased rapidly and peaked at 65% of their measurable potential abundance within 2 years ($P < 0.05$) following release and dominated all release plots throughout the study. Although population growth characteristics of black flea beetles were highly variable, the successful patterns in reducing the dominance of leafy spurge were fairly consistent. By 2004, foliar cover of leafy spurge on both release and nonrelease plots was significantly reduced compared to prerelease values. Foliar cover of grass and grasslike plants increased concomitantly with the reduction in leafy spurge dominance while cover of forbs on release and nonrelease plots remained consistently below noninfested values.

Resumen

Los escarabajos pulga "Negro" (*Aphthona lacertosa* y *Aphthona czwalinae*) y "Café" (*Aphthona nigriscutis*) están entre los agentes de control biológico más exitosos usados en el control y manejo del "Leafy spurge" a una escala relativamente grande en las Grandes Planicies del Norte. Los objetivos de este estudio fueron: 1) documentar las dinámicas de población de "Leafy spurge" en repuesta al control con escarabajos pulga "Negro" y "Café," 2) determinar el papel de las características del sitio seleccionado en el establecimiento y persistencia de los escarabajos, y 3) evaluar la respuesta general del a vegetación residente al control del "Leafy spurge." A fines de Junio del 1998, aproximadamente 3 000 insectos de cada especie fueron liberados en parcelas marcadas permanentemente en el noroeste de South Dakota y el sudeste de Montana. La abundancia de escarabajos, la densidad y cobertura foliar del "Leafy spurge" y la cobertura foliar de la vegetación residente se evaluaron anualmente de 1998 al 2004. Los escarabajos "Negros" se incrementaron rápidamente y en 2 años después de su liberación el pico de población estaba a un 65% de su abundancia potencial capaz de ser medida ($P < 0.05$) y dominaron todas las parcelas del estudio donde se liberaron. Aunque las características de crecimiento de la población del escarabajo pulga "Negro" fueron altamente variables, los patrones de éxito en reducir la dominancia del "Leafy spurge" fueron regularmente consistentes. Para el 2004, la cobertura foliar del "Leafy spurge" en las parcelas, tanto donde se liberaron los escarabajos y como en las que no se liberaron, fue reducida significativamente en comparación con los valores obtenidos antes de la liberación. La cobertura foliar de los zacates y plantas similares se incrementó con las reducciones de la dominancia de "Leafy spurge," mientras que la cobertura de las hierbas en la parcelas con y sin liberación de escarabajos permaneció consistentemente abajo de los valores de parcelas sin infestación.

Key Words: *Aphthona*, biological weed control, *Euphorbia esula*, Northern Great Plains

INTRODUCTION

Leafy spurge (*Euphorbia esula* L.) is an exotic plant species introduced into North America in the late 1800s that is now well established throughout much of the western United States and Canadian provinces. The success of this aggressive perennial is attributed to its ability to tolerate and exploit a variety of habitats and environmental conditions (Selleck et al. 1962; Nowierski et al. 2002). Heavy infestations of leafy spurge threaten the structural and functional integrity of many natural communities (Lym and Kirby 1987; Belcher and Wilson 1989; Trammell and Butler 1995; Butler and Cogan 2004). Many of the current control efforts emphasize conventional and

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integrated control programs utilizing herbicides, biological control with insects (Gassmann and Schroeder 1995), and behaviorally encouraging lambs to preferentially graze on leafy spurge (Walker et al. 1992).

Classical biological control involves the use of imported natural enemies to suppress or maintain populations of the target pest species below an economically or ecologically relevant threshold (Dahlsten 1986; Quimby et al. 1991; Piper 2004). Biological control is a useful tool for mitigating the impacts of exotic invasive plants; however, its application is not without risk (Carruthers and D'Antonio 2005, and articles contained in this special issue). Leafy spurge is considered a good candidate for biological control because of its growth habit, low density in its native habitat, and abundance of host-specific herbivorous insects (natural enemies) associated with this species in its native range (Harris et al. 1985; Gassmannn and Schroeder 1995; Gassmannn et al. 1996; Kirby et al. 2000). Biological control efforts on leafy spurge in the United States first began in 1965 with the introduction of the spurge hawk moth *Hyles euphorbia* L. (Lepidoptera: Sphingidae) (Rees and Spencer 1991). Between 1982 and 1996, six species of root-feeding flea beetles (Coleoptera: Chrysomelidae)—*Aphthona abdominalis* Duftschmid, *Aphthona cyparissiae* Koch., *Aphthona czwalinae* Weise, *Aphthona flava* Guillebeau, *Aphthona lacertosa* Rosenheim, and *Aphthona nigriscutis* Foudras—were collected from their native ranges, subjected to rigorous host specificity testing, approved, and then released in the United States (Rees and Spencer 1991; Hansen et al. 1997). To date, the most successful introductions have been those involving *Aphthona* spp. Adult flea beetles defoliate the aerial portion of the plant, while the larvae burrow into and feed upon the roots, usually causing considerable damage to the host plant (Rees et al. 1995).

Black (likely mixed populations of *A. lacertosa* and *A. czwalinae*) (Kalischuk et al. 2004) and brown flea beetles (*A. nigriscutis*) have extensive native distributions that encompass central and eastern Europe, Russia, central Asia, Eurasia, and eastern Siberia (Hansen 2004a, 2004b, 2004c). Introduced populations are now found throughout much of the Northern Great Plains (Fornasari 1996). Adults of these univoltine (1 generation per year) species begin to emerge in June, or when soil temperatures reach 25°C, and continue emerging through July (Fornasari 1996, Jackson 1997). Emerging adults quickly form aggregations for mating purposes and, within 4–16 days, the females begin oviposition, laying eggs on or just under the soil surface near the base of a host plant. The larvae emerge and immediately begin feeding upon the short roots and rootlets, which substantially impacts the water and nutrient uptake of the host plant (Gassmann and Schroeder 1995). The overall impact of this feeding, possibly mediated by interactions with soilborne pathogens (Caesar 2004) and influenced by plant genotype (Lym and Carlson 2002), is a reduction in plant stem density. Larval development continues through 3 instars by fall when they stop feeding to subsequently overwinter in the soil as mature larvae (Fornasari 1996). Feeding is resumed in the spring when soil temperature reaches approximately 15°C (Lym and Carlson 2002).

In June 1998, biological control assessment teams associated with The Ecological Area-wide Management of (TEAM) Leafy Spurge released black and brown flea beetles in select areas of Montana, North Dakota, South Dakota, and Wyoming (www.team.ars.usda.gov). Areas specifically selected for study represent the wide range of topographic, soil, vegetation, and landform situations typical of the region. The overall objectives of the biological control assessment effort were to document leafy spurge population dynamics in response to control by black and brown flea beetles, determine the role of selected site characteristics on establishment and persistence of the beetles, and evaluate the response of resident plants to control of leafy spurge by the two species of flea beetles. This paper evaluates the dynamics and trends of flea beetle populations, leafy spurge stands, and resident vegetation within the Montana and South Dakota study sites. Because of the relationships among the insects and their food source in their native range, we hypothesized that the insects would reduce the dominance of leafy spurge, as measured from estimates of cover and density, and that the resident vegetation would respond to this reduction in leafy spurge dominance through increased foliar cover.

MATERIALS AND METHODS

This study spanned a 6-year time interval from 1998 through 2004. Insects were initially released in 1998 and abundance was evaluated in mid-June to early July from 1999 through 2004. Baseline vegetation data were collected in June 1998 immediately prior to insect release. Vegetation data collected 2000 through 2004 were used to evaluate the response of the resident vegetation to any changes in leafy spurge cover and density. Vegetation data were not collected during the 1999 field season because of time and funding constraints.

Site Description

Two study sites, approximately 115 km apart, were selected in northwestern South Dakota (Harding County; lat 45°18′N, long 103°56′E) and southeastern Montana (Carter County; lat 45°53′N, long 104°21′E) within the larger TEAM Leafy Spurge study area. Both study sites are characterized by a semi-arid continental climate with long cold winters (−6°C long-term average low temperature) and variable summer conditions (20°C long-term average high temperature) with frequent hot days and cool nights (Johnson 1988; Vanderhorst et al. 1998). The majority of the precipitation recorded in Ekalaka, Montana, located 15 km west of the Montana study site, comes in late spring and early summer with a 30-year average of 438 mm (http://www.wrcc.dri.edu). Camp Crook, located 32 km north of the South Dakota study site, receives about 80% of the long-term average annual precipitation of 365 mm between April and September (http://hprcc.unl.edu/index.html). Vegetation of both study sites is dominated by mixed-grass prairie and shrublands that typify this part of the Northern Great Plains. The Montana study site (1050 m elevation) is topographically more diverse than the South Dakota study site (970 m elevation) and contains extensive stands of woodlands dominated by ponderosa pine (*Pinus ponderosa* Laws.). The South Dakota study site (6 200 ha) is managed by 3 ranchers and grazed by cattle and sheep while the Montana study site (2 600 ha) is located on 1 ranch and grazed by cattle.

Experimental Design

A total of 90 study plots were established in 1998 within the Montana (52 plots) and South Dakota (38 plots) study sites.

Each plot was characterized by leafy spurge infestations greater than 200 m^2 and permanently marked by primary and secondary markers located at the center of the infestation. Approximately 30 plots per study site were randomly selected during initial plot establishment to serve as potential release plots. At each plot, a physical survey was conducted, which included aspect, slope, and topographic position, to help ensure that the range of environmental conditions for the region was represented. Every attempt was then made to select nonrelease plots that matched release plots with respect to physical and biological attributes. In Montana, 32 release plots and 20 nonrelease plots were established, while 28 release plots and 10 nonrelease plots were established in South Dakota. The average distance between nonrelease plots and its nearest release plot, measured using a geographic information system, was 377 m for Montana (ranging from 100 to 934 m) and 311 m for South Dakota (ranging from 110 to 1 152 m).

During the 1999 field season, soil samples were collected from randomly selected plots in Montana (16 release and 12 nonrelease plots) and South Dakota (12 release and 7 nonrelease plots). Each sample consisted of a core (approximately 225 cm^3) taken from the top 5 cm of soil. Ten randomly located soil samples were collected from each plot and thoroughly mixed in a bucket. A subsample was then collected and analyzed for percentages of sand, silt, and clay, organic matter (OM), cation exchange capacity (CEC), potassium (K), sodium (Na), calcium (Ca), phosphorus (P), magnesium (Mg), nitrates (NO_3-N), and pH. Principal component analysis (PCA) was conducted to characterize interacting plot, biotic, and soil characteristics (PC-ORD 1999).

During the 2003 field season, when time and resources became available, additional plots were established in areas not infested by leafy spurge (hereafter referred to as noninfested). Noninfested plots were paired with infested plots (release and nonrelease) with regard to physical and biological site characteristics where possible. Plots were classified as noninfested if visible infestations of leafy spurge were at least 60 m from the plot, no leafy spurge cane (stem residue) was visible within the plots, and no flea beetles were present. Twenty noninfested plots were established in Montana and 9 in South Dakota.

A reference line was established perpendicular to the slope at each sample plot (release, nonrelease, and noninfested) with the survey marker serving as the center. Ten transects (5 upslope and 5 downslope) were placed at 30° intervals with the first transect beginning 30° from the reference line. A 5 × 4 m plot, with the primary survey marker in the center, and 6 randomly selected transects (3 upslope and 3 downslope) were used for estimating beetle abundance each field season (mid-June to early July) from 1999 through 2004. Each plot was sampled by sweeping each randomly selected transect and the 5 × 4 m center plot using 46-cm-diameter Bioquip sweep nets (canvas). Each transect consisted of 2 10-m sweeplines with a sweep at each pace (1 pace is approximately 1 meter). The first 10-m sweepline on each transect began 3 m from the primary marker, while the second sweepline began 13 m from the primary marker and ended 23 m from the primary marker, or until it exited the leafy spurge patch. Sampling the center subplot consisted of 2 5-m sweeplines, each positioned about 1 m from either side of the primary marker.

Following each sweepline, black and brown beetles were counted if the sample was visually estimated to contain < 100 individuals. A value of 100 was recorded if the observer judged the sample to consist of more than 100 beetles. Beetles were totaled for each plot and expressed as a percent of the maximum mean number of beetles that could be recorded per plot per sampling effort based on number of paces for each sweepline (percent abundance). For example, if the total number of sweeps on a given plot was 130 (20 for each of the 6 transects and 10 for the center subplot), and the maximum number of 100 beetles per sweepline was recorded, the maximum number of beetles that could be recorded for that plot would be 1 300 (100% abundance). The maximum number of beetles possible for each plot was reduced by 10 for each 1-m reduction in a sweepline caused by exiting the leafy spurge patch.

Data Analysis

Because brown beetles occurred infrequently, only black beetle abundance was used to evaluate the effects of slope on patterns of beetle abundance and spatial distribution. Mean peak black beetle abundance values on Montana release plots for 2000 and 2001 were averaged and then used to evaluate the potential effects of two slope classes ($n = 19$ for slopes < 11° and $n = 12$ for slopes ≥ 11°) using the Wilcoxon Two-Sample Test (SAS Release 8.1, 1999–2000) and found to be nonsignificant ($P = 0.51$). The effect of slope was not evaluated for the South Dakota plots because of the relatively uniform topography of the study area. The final analyses focused on evaluating the effect of year on black beetle abundance in release and nonrelease plots within each study site using standard analysis of variance procedures on ranked data (Conover and Iman 1981; SAS Release 8.1, 1999–2000). If significance was detected ($P \leq 0.05$), Bonferroni t tests were used to control the experiment-wise error rate.

Black beetle abundance profiles were created from the pool of Montana and South Dakota release plots that were continuously evaluated from 1999 through 2003 (attrition from a variety of land management activities over the 5-year period reduced the number of plots) using an Iterative Self-Organizing Data Analysis Technique (ISODATA). ISODATA is a nonhierarchical, divisive cluster analysis method effective for developing clusters with minimum internal variance (Ball and Hall 1967).

Density of leafy spurge and foliar cover of all plant species was estimated in each plot from eleven 20 × 50 cm (0.10 m^2) quadrats. One quadrat was placed within 1 m of the permanent marker and each of the remaining 10 quadrats were placed approximately 2.5 m from the marker along the 5 upslope and 5 downslope transects used for estimating beetle abundance. Foliar cover was estimated by placing each species occurring in the quadrat into 1 of 6 cover classes (1 = 0%–5%, 2 = 6%–25%, 3 = 26%–50%, 4 = 51%–75%, 5 = 76%–95%, and 6 = 95%–100%) (Daubenmire 1959). The midpoint of each cover class was used to sum cover by growth form for graminoids (grasses and grasslike species) and forbs for each quadrat. Distribution of mean foliar values by growth form did not meet assumptions of normality and homoscedasticity (Zar 1999); consequently, the effect of year on release and nonrelease plots was analyzed using standard analysis of variance (ANOVA)

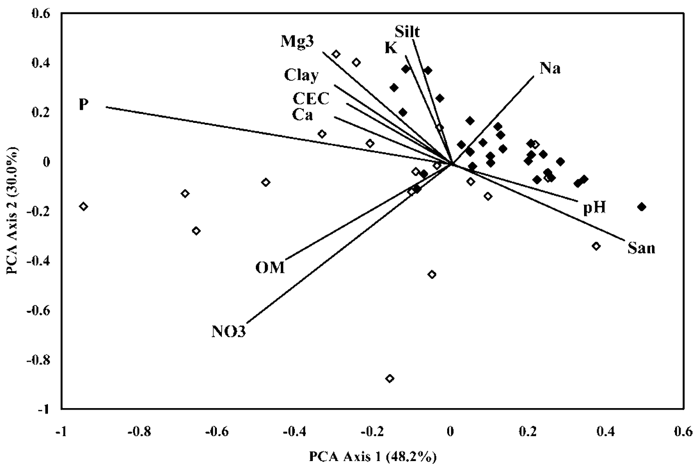

Figure 1. Principal Component Analysis of selected soil characteristics sampled from 47 randomly selected Montana (solid symbols) and South Dakota (open symbols) release and nonrelease plots.

procedures on ranked data (Conover and Iman 1981). If the ANOVA detected a significant difference ($P \leq 0.05$), means were separated using a Bonferroni *t* test.

RESULTS

Site Characterization

The area (m^2) of leafy spurge infestation at each plot was estimated using the average length of the 3 upslope ($\bar{a}$) and 3 downslope ($\bar{e}$) transects used to estimate beetle abundance in 1999 [area = ($\bar{a} \cdot \bar{e}$) $\cdot$ π]. A maximum patch area of 1 661 m^2 was recorded when the outer edge of the infestation was > 23 m from the permanent marker. Thirteen plots in Montana were located in infestations that were larger than 1 661 m^2. The average size of the infestations for the remaining 39 plots was 1 072 m^2 (ranging from 408 to 1 613 m^2). No infestations were evaluated within the South Dakota study area that equaled or exceeded the maximum patch size of 1 661 m^2. The average size of the infestations for the initial 38 plots established in South Dakota was 762 m^2 (ranging from 209 to 1 541 m^2). The total area of leafy spurge infestations used in this study was about 6.5 ha for Montana and 3.0 ha for South Dakota.

The results of the PCA on the 47 release and nonrelease plots selected for soil analysis are presented in Figure 1. PCA axes 1 and 2 cumulatively explain 78% of the environmental variation in soil texture and chemistry of the A-horizon, suggesting a moisture and productivity gradient. The overall distribution of South Dakota and Montana plots demonstrate very little overlap in this analysis. Montana plots appear to be scattered primarily along a soil texture gradient. Plots characterized by higher levels of sand are distributed along the right side of the first axis. In contrast, South Dakota plots are much more widely distributed and appear to be generally associated with the soil chemical properties that often characterize heavier clay soils. The distribution of South Dakota plots located on the left side of the ordination is characterized by higher levels of P, NO_3-N, and OM.

Beetle Abundance and Leafy Spurge Response

Black beetles dominated release plots in South Dakota and Montana throughout the study period (Table 1). Black beetles averaged 65%–68% of their potential abundance in Montana,

Table 1. Mean percent abundance of brown (*Aphthona nigriscutis*) and black (*Aphthona lacertosa*) beetles, mean percent leafy spurge foliar cover, correlation between cover and density, and mean leafy spurge density.[1]

		Nonrelease plots						Release plots				
		Brown	Black	Cover		Density		Brown	Black	Cover		Density
	n	% (±SE)			*r*	(stems · m^{-1})	*n*	% (±SE)			*r*	(stems · m^{-1})
Montana												
1998	20	—	—	56 (3.7) a[2]	0.83*	78 (8.3) a	32	—	—	52 (3.1) a	0.87*	70 (5.6) a
1999	20	T (0.0) a	0 (0.0) a	—	—	—	31	4 (0.7) ab	9 (1.1) a	—	—	—
2000	19	T (0.1) ab	4 (2.2) ab	45 (3.8) a	0.75*	79 (7.4) a	32	10 (2.2) a	65 (5.3) b	12 (1.8) b	0.90*	30 (3.5) b
2001	18	T (0.0) ab	19 (7.0) bc	37 (4.3) ab	0.75*	108 (13.4) a	31	4 (1.3) abc	68 (5.1) b	6 (1.5) c	0.91*	24 (4.2) bc
2002	16	1 (0.4) b	39 (10.3) c	18 (4.0) c	0.89*	73 (14.1) ab	28	5 (2.7) bc	34 (4.7) c	3 (0.7) c	0.96*	13 (2.7) c
2003	15	1 (0.5) ab	32 (8.1) c	20 (6.3) bc	0.61*	76 (20.9) ab	24	5 (2.9) c	24 (5.3) ac	10 (1.8) bd	0.68*	117 (30.3) a
2004	10	1 (0.4) ab	29 (11.1) c	8 (4.9) c	0.99*	26 (14.8) b	23	4 (2.0) c	20 (3.2) ac	5 (1.3) cd	0.74*	19 (5.8) bc
South Dakota												
1998	10	—	—	51 (7.8) a	0.78*	87 (10.1) ab	28	—	—	47 (2.7) a	0.63*	81 (7.3) a
1999	10	0 (0.0)	0 (0.0) a	—	—	—	28	2 (0.5) a	4 (0.6) a	—	—	—
2000	10	T (0.0)	4 (4.4) ab	27 (3.0) ab	0.62	56 (4.8) ab	28	3 (1.4) a	64 (5.6) b	16 (5.6) b	0.92*	40 (5.1) b
2001	10	T (0.0)	T (0.2) a	28 (7.8) abc	0.55	107 (17.8) a	28	2 (0.8) b	31 (6.9) a	6 (1.7) c	0.93*	28 (6.2) bc
2002	10	T (0.1)	10 (5.2) bc	11 (3.8) bc	0.98*	54 (16.8) ab	28	T (0.1) bc	29 (5.9) c	2 (1.0) d	0.87*	5 (2.5) d
2003	10	1 (1.5)	13 (10.9) c	25 (7.0) bc	0.67*	101 (18.5) a	27	T (0.0) c	11 (3.9) a	8 (1.3) c	0.43*	85 (13.0) a
2004	7	T (1.0)	2 (1.0) bc	7 (2.0) c	0.70	34 (12.8) b	20	T (0.0) c	5 (1.7) a	8 (2.4) c	0.96*	18 (4.7) c

[1]*n* indicates number of plots; *r*, correlation coefficient between cover and density; SE, standard error; *, $P < 0.05$; T, < 0.5.
[2]Column means among years within a study site followed by the same letter (i.e., a, b, or c) are similar ($P > 0.05$).

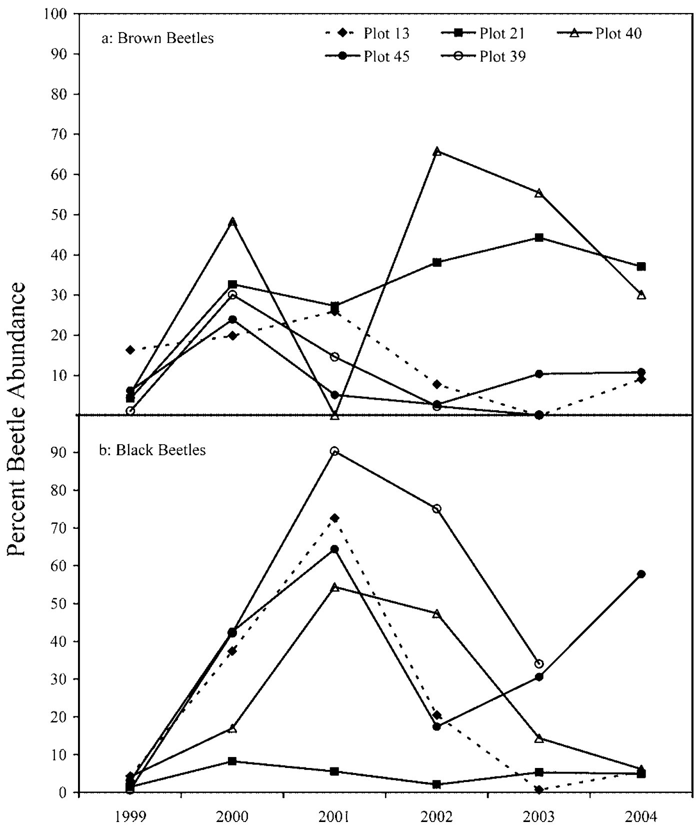

Figure 2. Population dynamics of **a**, brown and **b**, black flea beetles on 4 release plots in Montana (solid symbols) and South Dakota (open symbols).

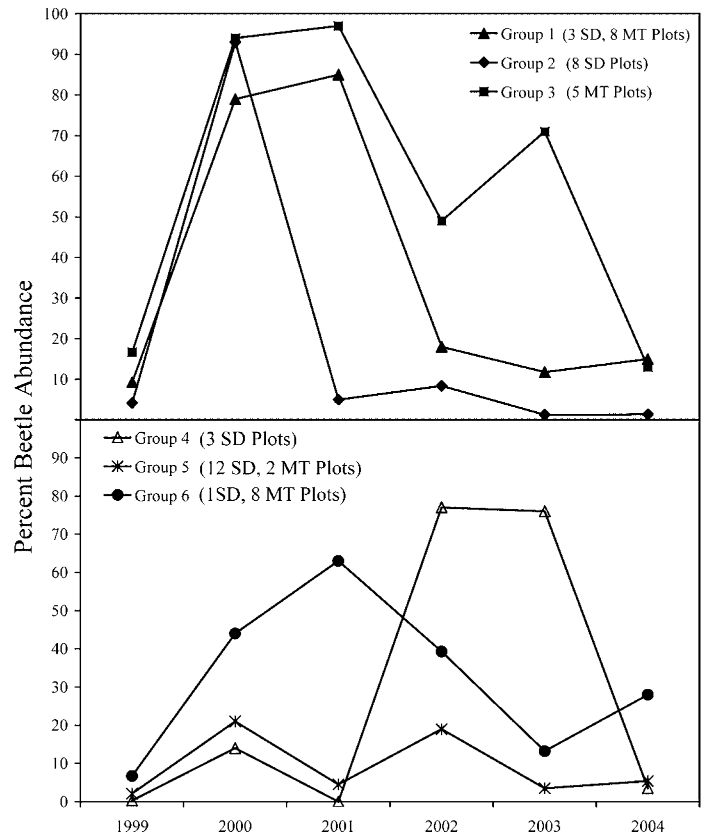

Figure 3. Black beetle abundance profiles for 6 groups identified using Iterative Self-Organizing Data Analysis Technique (ISODATA) cluster analysis of beetle abundance data. Groups are separated to reduce clutter.

based on sampling design, during peak beetle abundance (2000–2001), and peak abundance of black beetles in South Dakota occurred in 2000 at 64% of their measurable potential. Black beetle numbers quickly declined the year following peak abundance. Brown beetles were infrequent constituents on all release plots but tended to be more common in Montana. Stem density and foliar cover of leafy spurge decreased significantly following the rapid population growth of beetles (Table 1). Reductions in leafy spurge cover on release plots paralleled stem density through 2002, when correlation coefficients ranged from 0.87 to 0.96 for Montana, and 0.63 to 0.92 for South Dakota ($P < 0.05$). The lowest stem densities were recorded in 2002; however, densities increased 67% ($P < 0.05$) and 7% ($P > 0.05$) above 1998 values for Montana and South Dakota, respectively, in 2003. By 2004, mean foliar cover and density of leafy spurge on release plots were, respectively, 83%–90% and 73%–78% less than prerelease values recorded in 1998.

Brown beetles were rare on nonrelease plots in both study areas (Table 1). Black beetles on nonrelease plots in Montana demonstrated slow steady increases until abundance peaked in 2002. Patterns of reductions in leafy spurge cover demonstrated a corresponding decline on these plots, while stem densities remained fairly constant until 2004. Black beetles in South Dakota were first observed on nonrelease plots in 2000, but almost disappeared in 2001, and then returned the following 2 years. By 2004, foliar cover and stem densities on nonrelease plots were reduced to values similar to release plots.

The majority of brown beetles that were observed on release plots (Table 1) were primarily attributed to 4 plots in Montana and 1 plot in South Dakota (Fig. 2). The year of peak abundance varied considerably among the plots and ranged from 26 to 66% of potential. Highest brown beetle abundance occurred on Montana release plot numbers 21 and 40. With the exception of plot number 21, black beetles were abundant on all plots (Fig. 2). Plot number 13 differed from the other 4 plots in that the pattern of brown beetle abundance tended to parallel black beetle abundance on that site throughout the study period (Pearson Correlation Coefficient = 0.81, $n = 6$, $P < 0.05$).

Individual profiles that describe various patterns of growth in black beetle abundance were created using cluster analysis (ISODATA) on the 23 Montana and 20 South Dakota release plots that were consistently sampled from 1999 through 2004 (Fig. 3). The analysis identified 6 groups that accounted for 79% of the variation in beetle abundance over the evaluation period. Groups 1–3 describe populations that demonstrated the most rapid growth the first year. Peak black beetle abundance by 2000 in groups 1–3 was 77% of potential abundance, while peak abundance in groups 4–6 was 49% of potential abundance. Following peak beetle abundance, beetle numbers varied considerably among the 6 groups. Percent abundance in 2004 was lowest in groups 2, 4, and 5 (1%–5%) and highest in the remaining groups (13%–28%). Reductions in foliar cover and stem density of leafy spurge followed, for the most part,

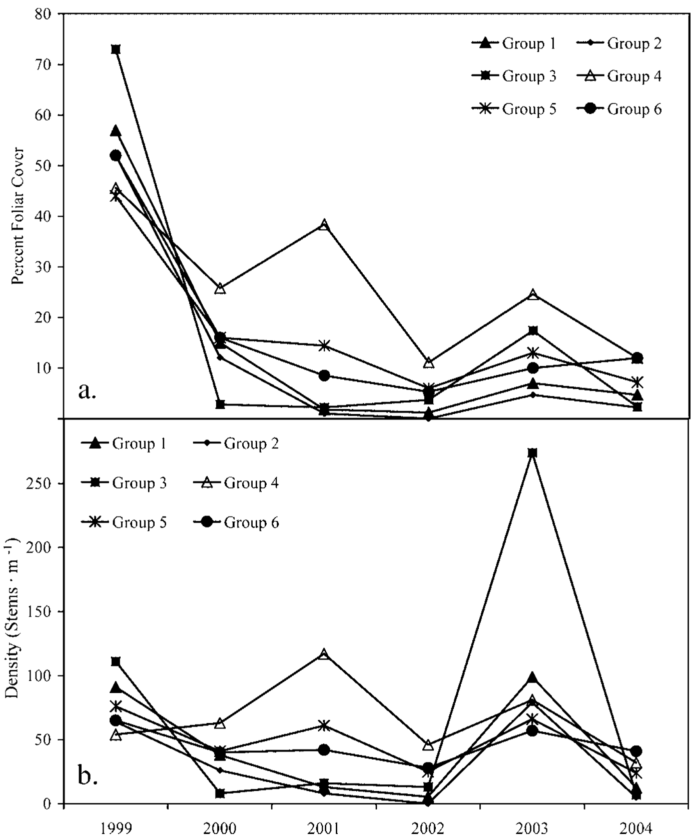

Figure 4. Percent **a**, leafy spurge foliar cover and **b**, stem density for 6 groups identified using Iterative Self-Organizing Data Analysis Technique (ISODATA) cluster analysis of beetle abundance data.

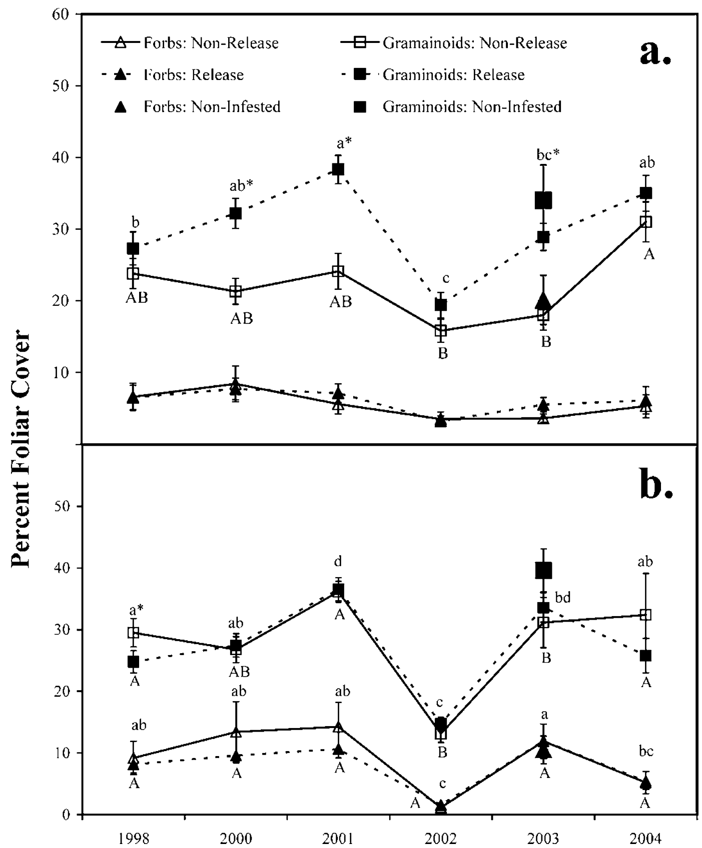

Figure 5. Percent foliar cover for graminoids (grass and grasslike; square symbol) and forbs (triangle symbol) on release (solid symbol with dotted lines) and nonrelease (open symbol with solid lines) plots, and noninfested plots for 2003 (single symbol) in **a**, Montana and **b**, South Dakota. Mean values with different letters indicate significant differences ($P < 0.05$) among years within release (lowercase) and nonrelease (uppercase) plots. *indicates differences ($P < 0.05$) in mean values between release and nonrelease plots within a sampling year.

parallel patterns (Table 1, Fig. 4). Stem densities increased in 2003 for all groups, especially group 3, which then decreased to 2002 values the following year. By 2004, mean foliar cover and density of leafy spurge was 86 to 96% less than 1998 values in groups 1–3. Foliar cover in 2004 for groups 4–6 was 74 to 84% less than 1998 values, while stem densities were reduced 37 to 68%.

Resident Vegetation Response

Graminoids dominated release and nonrelease plots for both Montana and South Dakota (Figs. 5a and 5b). Foliar cover contributed by graminoids on Montana release plots (Fig. 5a) increased nearly 60% ($P < 0.05$) between 1998 and 2001. Cover values for graminoids in Montana were lowest in 2002 for both release and nonrelease plots; however, cover increased in 2004 when values were 28 to 30% greater than 1998 values ($P > 0.05$). In 2003, foliar cover of graminoids on Montana noninfested plots was 29% greater than release plots and 98% greater than nonrelease plots.

In South Dakota (Fig. 5b), foliar cover of graminoids and forbs were similar between release and nonrelease plots throughout the study, with the exception of 1998. Significant reductions in cover occurred in 2002; however cover approached 1998 values the following year, which were similar to those recorded in noninfested plots. In 2003, forb foliar cover on release and nonrelease plots was similar throughout the study period in both study sites. Forb cover recorded on Montana noninfested plots was 218% greater than release plots and 377% greater than nonrelease plots in 2003 ($P < 0.05$). In contrast, forb cover on South Dakota release, nonrelease, and noninfested plots was essentially identical in 2003.

DISCUSSION

Flea beetles (*Aphthona* spp.) represent some of the more successful biological control agents used in the control and management of leafy spurge on a relatively large scale (Hansen et al. 1997; Lym 1998). Black beetles (*A. czwalinae* and *A. lacertosa*) and brown beetles (*A. nigriscutis*) represent 2 of the more common species introduced for controlling leafy spurge (Lym and Nelson 2000). In the present study, populations of these species were released into leafy spurge–infested sites that represented the wide variety of physical and biological conditions typical of the Northern Great Plains. Our releases of approximately 3 000 insects per species per plot was likely sufficient for potential establishment (Kalischuk et al. 2004). Because the insects do disperse naturally (Lym and Carlson 2002), and because unrecorded releases were made by landowners and managers, the results of the present study should be interpreted primarily in the context of evaluating responses among years within each group of release and nonrelease plots.

Hansen et al. (1997) considered flea beetle populations as established if 1 or more adults were collected at least once in at least 1 year following release. Collectable populations were those with a beetle-to-sweep ratio greater than 2.0, which was calculated by dividing the total number of beetles by the total number of insect sweeps, in at least 1 sampling visit. Using these criteria, introductions of black beetles produced established and collectable populations on the vast majority of release sites in South Dakota and Montana (Table 1: ratio > 2 when abundance $> 20\%$). The average sweep-to-beetle ratio for black beetles introduced in this study was < 2 ($< 20\%$ abundance) only during 1999 and 2003 in South Dakota and 2004 in Montana. During periods of peak abundance (2000 and 2001), the average value was > 6.0 ($> 60\%$ abundance). The ratio for brown beetles averaged < 1 ($< 10\%$ abundance) on release plots throughout this study.

Nowierski et al. (2002) reported that the black beetle (*A. czwalinae* and *A. lacertosa*), in its native range in Europe, preferred or tolerated moister sites with higher levels of silt, clay, and plant productivity, while the brown species (*A. nigriscutis*) tended to occur more commonly on drier sites with moderate levels of plant productivity. The results of the PCA used in this study suggest considerable environmental variation in the distribution of release and nonrelease plots similar to the study sites evaluated in Europe (Nowierski et al. 2002). Soil characteristics for sites selected as release or nonrelease generally fell well within the range of optimum conditions for leafy spurge and black flea beetles. Although the Montana release plots generally fell along a clay to sand soil texture gradient, sites with soils considered too sandy for the establishment of black beetles were not observed (maximum sand content was 85%; data not shown).

Although black and brown beetles were released in equal numbers, black beetles were far more abundant than brown beetles in both study sites throughout this investigation. These results are substantially different from those reported by Larson and Grace (2004). In their study, populations of both black and brown beetles were widely introduced into Theodore Roosevelt National Park beginning in 1987. By 1999, both species were well-established in a wide variety of vegetation types throughout the Park. In that study, black beetle abundance was not found to be significantly greater than brown beetles in any vegetation type, despite the overall greater number of releases thought to consist primarily of black beetles. Jonsen et al. (2001) reported significantly higher immigration probabilities for brown beetles in Canada as compared to black beetles, especially in a grassland-dominated landscape. They suggested that the larger wings of brown beetles, compared to black beetles, may play a major role in the higher dispersal patterns. Jacobs et al. (2001) indicated that the number of leafy spurge flowering stems may be an important factor that influences the successful establishment of brown flea beetles, while Lym (1998) reported that establishment was reduced if overall stem densities were greater than 320 stems $\cdot$ m^{-2}. We did not separate vegetative stems from flowering stems while estimating stem densities; however, stem counts from individual plots were less than 200 stems $\cdot$ m^{-2}. Based on the limited number of plots where significant numbers of brown flea beetles were recorded in our study, the insects appeared to be restricted to the few plots high in sand content similar to the dry, coarse-textured sites reported by Nowierski et al. (2002).

Black beetles exhibited considerable variation in abundance profiles throughout the sampling period. Kalischuk et al. (2004) reported that black beetles released in Alberta, Canada, with the greatest potential for population growth were on sites south of Calgary that were characterized by the most rapid accumulation of degree days. Observed differences in the initial rate of growth of black beetles in our study were almost evenly divided among plots in South Dakota and Montana. Because our release sites are located well south of Calgary (51°N vs. 45°N) population growth was not likely limited by the accumulation of degree days on a geographic scale. Local site characteristics such as slope, aspect, and vegetation type that may influence microclimate variability did not appear to play a major role in the establishment and growth patterns of black beetles or in their ability to effectively and quickly reduce the dominance of leafy spurge. Despite the varied growth patterns of the insects, percent black beetle abundance among the 6 groups (Fig. 3) at the end of the experiment averaged 11% (ranging from 1%–28%) while average foliar cover and density of leafy spurge variably and respectively converged to 7% (ranging from 2%–12%) and 20 stems $\cdot$ m^{-2} (ranging from 5–41 stems $\cdot$ m^{-2}). The observed variations in flea beetle abundance and declining leafy spurge populations may be a reflection of a combination of interacting factors unrelated to the flea beetles that include plant genotype (Lym and Carlson 2002), initial stem density (Larson and Grace 2004), and the presence or absence of possible synergistic interactions of the insects with soilborne plant pathogens (Caesar 2004).

Cover and especially density of leafy spurge increased the year following peak control, while beetle abundance continued to decline. No attempts were made to quantitatively separate established plants from emerging seedlings during estimates of stem density; however, the vast majority of leafy spurge plants counted in 2003 for both study sites were seedlings (J. L. Butler, unpublished data, June 2003). A fairly well-developed root system, which is not likely to be provided by leafy spurge seedlings, is required for flea beetle larval development (Fornasari 1996). Consequently, insect abundance continued to decline even though leafy spurge appeared to be recovering on some release sites. However, beetle numbers increased in 2004 on some sites (Fig. 3), suggesting a possible feedback mechanism wherein insects may track their food source (Larson and Grace 2004). This pattern may also be an artifact of releasing relatively large numbers of insects in a small area that was repeatedly and intensively sampled (i.e., the "bomb blast" often observed following release of flea beetles). Because the insects more or less naturally spread to nonrelease plots in a dispersed fashion, a feedback mechanism would not be as apparent on these sites. If a feedback mechanism does exist, then the time frame between the seedling stage and mature stage of leafy spurge may create a lag phase in beetle population dynamics on release plots (for example, Group 3; Figs. 3 and 4).

Black beetles first appeared on most nonrelease plots in Montana in 2000 and the beetle-to-sweep ratio exceeded 2.0 by 2002. Neither black nor brown beetles appeared to produce collectible populations on nonrelease plots in South Dakota (percent abundance < 20). Several interacting factors probably contributed to low beetle abundance in South Dakota compared to Montana. First, infestations of leafy spurge in South Dakota were typically much smaller and more widely distributed than those found on the Montana study site, which likely reduced the potential for natural dispersal of the insects (Jonsen et al. 2001). Second, because of the overall high infestation level within the Montana study site, several off-plot releases were made by landowners and managers in 1999 and 2000, increasing the probability that insects would eventually disperse into nonrelease plots. Finally, the difference in leafy spurge cover values between release and nonrelease plots in 2000 and 2001 probably reflects a role played by sheep grazing in reducing leafy spurge on the South Dakota plots. By the time beetles on South Dakota release plots reached peak abundance (2000), beetles were rare on nonrelease plots, while leafy spurge cover and stem density on nonrelease plots were, respectively, 50% and 29% of prerelease values.

The increase in cover for the resident vegetation immediately around Montana release plots closely corresponded to the reductions in leafy spurge cover and density. Within 3 years of the insect releases (2001), leafy spurge cover and density were respectively reduced 88% and 66%, while foliar cover of graminoids increased 60% compared to prerelease values. However, foliar cover of graminoids on release plots recorded the following year decreased to values similar to nonrelease plots and significantly below prerelease values. The observed pattern of declining graminoid cover may be a reflection of increased grazing pressure on release plots (Kirby et al. 2000) in addition to underlying vegetation community dynamics. Lym and Kirby (1987) reported that cattle grazing behavior was negatively impacted when leafy spurge stem densities were greater than $100/m^2$. Such a threshold level probably varies with the physical and biological characteristics of the site. However, reducing stem densities below a critical threshold would also likely increase the probability of livestock grazing (Kirby et al. 2000). In our study, grazing pressure probably increased on release sites immediately following the significant reductions in leafy spurge cover and density that occurred in 2002. Further, precipitation was 44% less than the long-term average in 2002 for both the Ekalaka and Camp Crook recording sites (http://www.noaa.gov/climate.html), which likely compounded the effects of grazing on the low foliar cover recorded for graminoids. The dry conditions observed in 2002 were also likely contributors to the reduction in leafy spurge cover and density on all study plots. The gradual reduction in leafy spurge characteristic of Montana nonrelease plots is reflected in the gradual but significant increase in graminoids by 2004. At this point, cover on release and nonrelease plots was comparable to noninfested plots.

Changes in foliar cover of graminoids and forbs over time on South Dakota release and nonrelease plots paralleled each other throughout the study. Similar to trends observed on release plots in Montana, graminoid cover on South Dakota release plots was significantly greater in 2001 compared to 1998. However, graminoid cover also increased on South Dakota nonrelease plots during that same time period ($P > 0.05$), when foliar cover of leafy spurge was 78% less than 1998 values. The lack of beetles on nonrelease plots suggests that sheep grazing may have been sufficiently heavy to reduce leafy spurge cover while not significantly interfering with the recovery of resident vegetation. Soilborne pathogens, acting singly or in combination with sheep grazing and flea beetle activity, may have also played a role in the reduction of leafy spurge (Caesar 2004). As was observed in Montana, graminoid cover was significantly reduced in 2002 and then recovered to prerelease and noninfested values by 2003.

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

Successful establishment of black flea beetles occurred on a wide variety of grassland and shrubland sites typical for the region under a variety of land management strategies. Although population growth characteristics of black beetles were highly variable across the range of study plots, the observed patterns of reduced leafy spurge dominance were fairly consistent. Brown beetles were infrequent constituents on the vast majority of plots and appeared to be restricted to locations characterized by drier, coarser (sandy) soils. The reduction in leafy spurge cover appeared to stimulate seedling recruitment, which we observed to correspond with a noticeable drop in beetle abundance. Should insect populations decline below some unknown threshold because of low abundance of mature leafy spurge plants (high numbers of seedlings), long-term control may require re-releases of insects. In contrast, the observed dynamics in leafy spurge and flea beetles may be a short-term phenomenon that is part of a long-term population cycle that ultimately results in equilibrium between the biological control agent and its food source.

While graminoid cover increased following reductions in the dominance of leafy spurge, forb cover in Montana on release and nonrelease plots remained well below levels observed on noninfested plots and changed very little throughout the study. Infestations of leafy spurge appear to have a strong filtering effect on composition and structure of impacted plant communities (Butler and Cogan 2004). Results of that study in Theodore Roosevelt National Park, North Dakota, indicated that 70% of the 30 species identified as sensitive to infestations of leafy spurge were forbs (Butler and Cogan 2004). The lack of change in forb cover coupled with the significant increase in graminoid cover suggests that loss of forbs may become, from a practical perspective, a long-term phenomenon without intensive management. Long-term monitoring is necessary to fully evaluate the population dynamics and inter-relationships between leafy spurge and flea beetles, and the recovery status of the resident vegetation.

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