Potential Impact of Two *Aphthona* spp. on a Native, Nontarget *Euphorbia* Species

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Abstract

Flea beetles (*Aphthona* spp.) are biological control agents introduced from Eurasia to reduce the cover and density of leafy spurge (*Euphorbia esula* L.). The potential for the introduced beetles to use alternate hosts for feeding and development in North America is slight; however, it is possible. Species at highest risk are native species closely related, ecologically and taxonomically, to leafy spurge. A native spurge, *Euphorbia brachycera* Engelm. is consubgeneric and sympatric with leafy spurge throughout the northern Great Plains of the United States, and was not included in prerelease host-specificity testing for *Aphthona nigriscutis* or *Aphthona lacertosa*. The objective of this study was to evaluate the actual and potential ecological overlap among leafy spurge, flea beetles, and *E. brachycera*. Wide-ranging and intensive field surveys indicate that *E. brachycera* is found well within the range of leafy spurge and flea beetles. *E. brachycera* occurs infrequently, in low densities, in areas with a high percentage of bare ground, and with a root system dissimilar to leafy spurge. Flea beetles released directly into populations of *E. brachycera* failed to persist beyond a single field season and plants showed no evidence of feeding by beetles. Our results suggest that the potential for flea beetles to host-shift is low due to differences in growth habit and root morphology between *E. brachycera* and leafy spurge.

Resumen

Los escarabajos "Pulga" (*Aphthona* spp.) son agentes de control biológico introducidos de Eurasia para reducir la cobertura y densidad de "Leafy spurge" (*Euphorbia esula* L.). El potencial de los escarabajos introducidos para usar hospederos alternativos para alimentarse y desarrollarse en Norteamérica es bajo, sin embargo, es posible. Las especies con mayor riesgo son especies nativas cercanamente relacionadas ecológica y taxónomicamente al "Leafy spurge." Un "Spurge" nativo *Euphorbia brachycera* Engelm. es consubgenerico y simpatrico con el "Leafy spurge" a través de las Grandes Planicies de Estados Unidos de América y no fue incluido en las pre-liberaciones de prueba para determinar las especificidad de los hospederos del *Aphthona nigriscutis* o *Aphthona lacertosa*. El objetivo de este estudio fue evaluar el traslape ecológico actual y potencial entre el "Leafy spurge," los escarabajos "Pulga" y *E. brachycera*. Muestreos de campo amplios e intensivos indican que *E. robusta* se encuentra dentro del rango del "Leafy spurge" y de los escarabajos "Pulga." *E. brachycera* ocurre infrecuentemente, en bajas densidades, en áreas con alto porcentaje de suelo desnudo y con un sistema radical diferente al del "Leafy spurge." Los escarabajo "Pulga" liberados directamente en poblaciones de *E. brachycera* fracasaron en persistir mas allá de una sola estación y las no plantas mostraron evidencia de que hayan sido consumidas por los escarabajos. Nuestros resultados sugieren que el potencial de los escarabajos de ocupar otros hospederos es bajo debido a diferencias en el hábito de crecimiento y morfología de la raíz entre *E. brachycera* y "Leafy spurge."

Key Words: ecological separation, ecological risk, leafy spurge

INTRODUCTION

In an effort to control invasive plants such as leafy spurge (*Euphorbia esula* L.) and Canada thistle (*Cirsium arvense* [L.] Scop.), many integrated pest management plans include the use of biological control agents in conjunction with herbicides. While biological control is often considered safer (Van Driesche 1994) than chemical control, it is not without ecological risk. Potential risks specific to the biological control of weeds include food-web alterations (Pearson and Callaway 2003) and host-shifting of biocontrol agents onto cultivated and/or native plants (Pemberton 1984). Organisms most threatened by host-shifting of biocontrol agents are native plants most closely related ecologically and taxonomically to the target species (Pemberton 2000). Additionally, as the target population declines, the potential impact on nontarget species being used as forage and as a developmental host increases.

Use of nontarget species by biocontrol insects has been documented for several native plants. Louda and O'Brien (2002) reported that the seed-feeding weevil *Larinus planus* Fabricius, established in the United States and Canada for the control of Canada thistle, often foraged on the native Tracy's thistle, *Cirsium undulatum* (Nutt.) Spreng. var. *tracyi* (Rydb.)

Table 1. Site name, site abbreviations, date of vegetation survey, topographic characteristics, and location for each study site.

Site	Site abbrev.	Date of survey (2002)	Slope	Elevation (m)	Aspect	Latitude	Longitude
Wind Cave 1	WICA1	17 June	14°	1170	south	43°35′29.5″	103°23′29.2″
Wind Cave 2	WICA2	29 June	8°	1164	southeast	43°35′14.3″	103°23′35.4″
Lander 1	LAN1	21 June	16°	1841	northeast	42°48′06.1″	108°47′43.6″
Lander 2	LAN2	23 June	20°	1646	west	42°50′11.9″	108°34′02.1″
Lander 3	LAN3	24 June	2°	2060	southeast	42°36′58.1″	108°26′43.9″
Lander 4	LAN4	25 June	9°	2054	south	42°37′00.4″	108°26′56.8″
Black Hills 1	BH1	12 July	2°	1408	southeast	43°39′21.5″	104°01′41.8″
Black Hills 2	BH2	13 July	5°	1408	south/southwest	43°39′21.9″	104°01′44.6″

Welsh. Nontarget feeding by a biocontrol agent was discovered in several populations of cacti (*Opuntia* spp.) in Florida, some of which are rare and endangered (Stiling and Moon 2001). While the cactus-feeding moth, *Cactoblastis cactorum* Bergroth, was not released in the conterminous United States, it has traveled to Florida from the Caribbean naturally or on cacti imported for the horticultural market (Pemberton 1995).

Prior to intentional release, potential biocontrol agents undergo rigorous host-specificity testing including adult feeding preferences and oviposition (Gassmann et al. 1996). Despite these laboratory tests, there have been cases in which an agent has been identified as host-specific in the laboratory (Zwölfer and Harris 1984) but field evidence demonstrated otherwise (Turner et al. 1987; Louda et al. 1997; Louda et al. 2003). However, not all host specificity tests can accurately predict ecological interactions once the agent has been introduced into a new environment (Pearson and Callaway 2003). Furthermore, few biocontrol programs include post-release field tests (Simberloff and Stiling 1996). Specifically, no published studies involving leafy spurge have been found that simultaneously addressed the ecological relationships among biocontrol agents, the intended target, and potential nontarget species. Dissimilar ecological relationships among principal species may play a subtle but important role in reducing the potential nontarget impacts.

Leafy spurge is an exotic, invasive weed that infests over 5 million acres in 35 states in the western and northern United States and the Canadian prairie provinces (TEAM Leafy Spurge, http://www.team.ars.usda.gov). Heavy and widespread infestations have caused damage to a variety of ecosystems, cost millions of dollars due to lost productivity, and increased ecological damage from the heavy use of herbicides (Bangsund and Leistritz 1991). Biocontrol programs were developed and incorporated into integrated pest management strategies for leafy spurge because of the high cost and environmental concerns of using herbicides (Lym 1998).

In the northern United States, the brown flea beetle (*Aphthona nigriscutis* Foudras) and the black flea beetle (*Aphthona lacertosa* Rosenhauer) have demonstrated considerable and widespread success in reducing leafy spurge (McClay et al. 1995; Kirby et al. 2000; Butler et al. 2006). Prerelease research was conducted regarding possible nontarget effects of *Aphthona* spp. on North American spurges, including species in the same subgenus (*Esula*) as leafy spurge. However, the native spurge *Euphorbia brachycera* Engelm. (formerly *Euphorbia robusta*), a consubgener, is conspicuously missing from published research involving *A. nigriscutis* and *A. lacertosa* (e.g.,

Pemberton 1984; Gassmann et al. 1996; Rees et al. 1996). *E. brachycera* is similar to leafy spurge in its perennial life span and its ecological range; the range of *E. brachycera* covers the northern Great Plains, from Idaho to Minnesota, and south into Nebraska, New Mexico and Arizona (McGregor 1986; Larson and Johnson 1999; USDA NRCS PLANTS Database 2005, http://plants.usda.gov/).

As a result of the success of flea beetles, their range is expanding to cover a larger part of the range inhabited by *E. brachycera*. This overlap of the target species, the biocontrol agent, and *E. brachycera* makes *E. brachycera* a possible candidate for *Aphthona* spp. feeding and development. While none of the *Euphorbia* spp. under consideration for protection occurs in areas currently inhabited by leafy spurge and flea beetles, the distribution of *E. brachycera* is extensive and it could potentially serve as a bridge to threatened *Euphorbia* species.

The objective of this study was to evaluate the potential impact of introduced flea beetles on *E. brachycera*. Specifically, the goal was to use ecological similarities and differences among *E. brachycera*, leafy spurge, and flea beetles to help elucidate any threat to the native, nontarget plant that may be posed by release of these biocontrol agents. A secondary goal was to assess the use of *E. brachycera* as a food source and developmental host by *A. lacertosa* in a field setting.

MATERIALS AND METHODS

Extensive reconnaissance conducted during the 2000 and 2001 field seasons covered the general area included in the research efforts of TEAM Leafy Spurge (The Ecological Area-wide Management of Leafy Spurge [http://www.team.ars.usda.gov]); specifically, selected parts of central Wyoming, western South Dakota and North Dakota, and southeastern Montana. From these areas, 8 study sites were selected based on having populations of *E. brachycera* greater than 20 individuals within a 1-ha plot and subsequently sampled in June and July 2002. All sites have continental climate, with the majority of precipitation occurring April through September (Young 1981; Ensz 1990).

Four sites were located in the Red Valley of the Black Hills of South Dakota—2 in Wind Cave National Park (Wind Cave NP) (WICA1 and WICA2) and 2 in the Elk Mountains on land managed by the Black Hills National Forest (Black Hills NF) (BH1 and BH2) (Table 1). The sites in Wind Cave NP are typified by mixed grass prairie, dominated by little bluestem

(*Schizachyrium scoparium* [Michx.] Nash), western wheatgrass (*Pascopyrum smithii* [Rydb.] A. Löve), and grama grasses (*Bouteloua* Lag.) (nomenclature follows NRCS PLANTS Database 2006). The sites on the Black Hills NF are dominated by ponderosa pine (*Pinus ponderosa* P. & C. Lawson) with a mixed grass understory, comprised of species similar to those in Wind Cave NP. Soils in the Red Valley are weathered from gypsum and gypsiferous siltstone and are red to reddish-brown in color. The soils are low in fertility and organic matter with low available water capacity, moderate permeability, and medium to rapid runoff (Ensz 1990). Sites were grazed by bison (NP), cattle (NF), and wildlife.

The remaining 4 sites were located near Lander, Wyoming (LAN1, LAN2, LAN3 and LAN4), in south-central Fremont County at the base of the Wind River Range, on private property and land managed by the Bureau of Land Management (BLM). All 4 sites are typified by sagebrush steppe. Dominant species include big sagebrush (*Artemisia tridentata* [Nutt.] ssp. *wyomingensis* [Beetle & Young]) and bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Löve). The 4 study sites are characterized by 2 soil series, Sinkson and Thermopolis (Young 1981). Sinkson soils are well drained and often very deep with moderate permeability. The soil water holding capacity is high, with moderate runoff that results in moderate to highly erodible soils. Thermopolis soils are typically upland soils that are shallow and well-drained, with moderate permeability and low water holding capacity. Sites were grazed by cattle (BLM only) and wildlife.

Once a site was selected, every *E. brachycera* plant was marked with a pin flag. Because of the low number of plants at each site, it was possible to visually inspect every *E. brachycera* plant and evaluate it for the presence of flea beetles and insect feeding damage. Evaluation occurred during site reconnaissance in early summer and on the survey date (Table 1). No flea beetles were found on any of the *E. brachycera* plants.

At each site, a 100 × 50 m macroplot was established (except for LAN1, where a 100 × 100 m macroplot was used) with the center of each plot placed in the center of the *E. brachycera* population. Twenty-five 4-m^2 subplots were randomly placed in each of the 4 quadrants of the macroplot for a total of 100 randomly selected subplots per site. Each subplot was sampled for the presence or absence of *E. brachycera*, and then subdivided into 4 1-m^2 microplots. One microplot was randomly selected per subplot and sampled for foliar cover by species, litter, and bare ground using the following six cover classes: 1) 0%–5%, 2) 5%–25%, 3) 25%–50%, 4) 50%–75%, 5) 75%–95%, and 6) 95%–100% (Daubenmire 1959). The midpoint value of each cover class was used to calculate mean cover for each microplot. Additionally, varying numbers of subjectively selected 1-m^2 microplots with *E. brachycera* present were sampled so that each macroplot had 25 microplots with *E. brachycera*.

Vegetation analysis involved hierarchical clustering using Euclidean metrics for distance measures, and group linkage followed Ward's method (PC-ORD 1999). The results were used for grouping the microplots with most similar vegetation characteristics, with each site ultimately having 4 groups. After all the microplots were grouped, the microplots within each group were divided into 2 categories: "absent," which contained no *E. brachycera* plants, and "present," which all

contained *E. brachycera*, regardless of whether the microplot was randomly or subjectively selected. For each site, species of importance were determined by selecting species with a high frequency of occurrence and/or high average percent cover value using dissimilarity analysis (Dyer 1978).

Ten 0.5-kg soil samples (5 inside and 5 outside the macroplot) were randomly collected at each site, with the exception of LAN1, where 8 samples (4 inside and 4 outside the macroplot) were collected, and LAN2, where 4 were collected (all within the macroplot). At each location, the surface duff was removed and samples were collected from the top 15 cm of the soil profile. Each soil sample was analyzed individually at the South Dakota State University Soil Testing Laboratory, Brookings, South Dakota. Samples were analyzed for 30 chemical and physical characteristics.

Soil data were analyzed using principal component analysis (PCA) to evaluate soil characteristics that could potentially be used to predict the presence or absence of *E. brachycera*. In the course of performing PCA, the number of variables was reduced to a level for which a PCA could be reliably estimated (Hatcher 1994; McCune et al. 2002). The final analysis was performed using the following 11 elements: exchangeable bases of calcium, magnesium and potassium, cation exchange capacity, percentage of exchangeable cations of calcium, magnesium and potassium, saturated paste extracts of calcium and magnesium, saturated paste extract for electrical conductivity (a measure of soil salinity), and gypsum. Since flea beetles are sensitive to soil texture, a separate PCA was conducted using the percent of sand, silt, and clay of the soil samples.

To evaluate the potential use of *E. brachycera* by flea beetles in a field setting, 2 sites in South Dakota (BH1 and BH2) were selected to serve as beetle release sites. On 17 July 2002, beetles were collected near Ekalaka, Montana (lat 45°53′N, long 104°23′W), and approximately 300 *A. lacertosa* were released on each of 20 (BH1) and 14 (BH2) marked *E. brachycera* plants. An additional 20 (BH1) and 14 (BH2) *E. brachycera* plants were located and marked at the opposite end of the macroplot to serve as nonrelease control plants. The low population density of *E. brachycera* at each site limited the number of plants that could be included in the experiment. We assumed limited dispersal because of the gregarious nature of the insects (McClay et al. 1995) and the wide spacing of *E. brachycera* plants. LAN1 was considered to have beetles present because the site is immediately adjacent to a population of leafy spurge controlled by flea beetles (*A. nigriscutis*) released in 1992 by the Fremont County Weed and Pest Department (L. Baker, personal communication, 2003). Additionally, in 1999, beetles and feeding damage were observed on the *E. brachycera* population at LAN1 (Baker et al. 2004).

Thirty *E. brachycera* and 30 leafy spurge plants were collected from LAN1 on 17 April 2003. Entire *E. brachycera* plants, including the tap root and as much of the leafy spurge lateral root system as possible, were collected and placed in plastic containers for transport to a South Dakota State University greenhouse. Mesh insect netting was placed in the bottom of each container to cover drainage holes and over the tops of the containers to contain emerging beetles. Ten and 7 of both release and nonrelease plants were randomly selected and collected on 20 April 2003 from BH1 and BH2, respectively, using the same methods. All plants were kept alive in a

greenhouse environment until beetle emergence. On 11 June 2003, after full beetle emergence, plants were separated from the soil and inspected for beetle feeding damage and presence of insects. Soil from the leafy spurge plants was sieved through a #60 sieve to detect beetles in the soil. Biomass samples from all *E. brachycera* plants collected from BH1, BH2, and LAN1 were retained. Above- and belowground material for each plant was dried separately at 60°C for 48 hours and weighed.

A Wilcoxon rank-sum test for 2 samples (Cody and Smith 1997; Zar 1999) was used to test the null hypotheses of no differences ($P > 0.05$) in above- and belowground biomass between release and nonrelease plants (SAS version 8.1, 1999–2000). This test was employed because of the small sample size and uneven number of samples. Biomass samples were compared at the site level between release and nonrelease plants using aboveground, belowground, and total (above- and belowground combined) biomass. The CATMOD (CATegorical data MODeling) procedure in SAS (Stokes et al. 2000) was used to test the null hypothesis of no differences in plant mortality between *E. brachycera* specimens collected and kept in the greenhouse, plants left in the field, plants subjected to beetle releases (release), and those without beetle releases (nonrelease). Each main effect was evaluated, as well as possible interactions between treatments.

RESULTS AND DISCUSSION

E. brachycera occurred infrequently and in low densities at all sites. WICA1 had the lowest frequency of *E. brachycera* with 4% of the random subplots containing plants, while WICA2 had the highest frequency of plants (19%). *E. brachycera* densities ranged from 10 plants $\cdot$ ha^{-1} at WICA1 to 73 plants $\cdot$ ha^{-1} at LAN2 (data not shown). Concomitantly, foliar cover of *E. brachycera* was considerably less than 1% at all sites. Furthermore, the groups created from the cluster analysis revealed that the amount of bare ground served as the largest source of (dis)similarity between vegetation groups and as the primary indicator of *E. brachycera* presence (Table 2). The 2 plant species that contributed the most to the (dis)similarity at each site are listed in Table 2, along with the foliar cover values for *E. brachycera* in each group. Plant species serving as dominants varied at each site, suggesting considerable ecological amplitude of *E. brachycera* with respect to plant associations.

At each of the sites, with the exception of one group at BH2, the vegetation group with the largest number of subplots with *E. brachycera* had the highest percentage of bare ground (mean of 51% for *E. brachycera* "present" plots; 42% for "absent" plots). In contrast to the low density observed for *E. brachycera*, leafy spurge can form dense monotypic stands with stem densities capable of reaching over 350 stems $\cdot$ m^{-2} (Mundal et al. 2000). Stem density of leafy spurge is often correlated with foliar cover (Butler et al. 2006). Consequently, it is possible for a stand of leafy spurge with high stem density to reach 100% foliar cover, exposing very little bare ground. The ability of leafy spurge to form dense, monotypic stands is due, in part, to the extensive root system and the ability to form adventitious buds along the entire length of the root (Messersmith et al. 1985). *E. brachycera* does not have such a root

system; rather it forms a single, heavy tap root, with occasional root hairs, from which a single plant arises.

E. brachycera was found to occupy habitats similar to those invaded by leafy spurge. Populations of *E. brachycera* were found in areas with minor disturbance, e.g., erosive, exposed soils and grazed areas in shrublands (LAN1, LAN2, LAN3, and LAN4) and woodlands (BH1 and BH2), as well as in relatively undisturbed native grasslands (WICA1 and WICA2). Leafy spurge has been documented in the same vegetation types, and occurs with many of the same species that were found at the study sites (Jacobs et al. 2001; Baker et al. 2004; Butler and Cogan 2004; Larson and Grace 2004). Additionally, *E. brachycera* demonstrated an affinity for coarser textured soils in a fashion similar to leafy spurge (Selleck et al. 1962). Soil texture was quite variable but generally remained consistent within sites. Although the 72 soil samples fell into 7 texture classes, soils at sites were predominately moderately fine to medium textured (Table 3). Seventy-one percent of the *E. brachycera* population occupied medium to moderately coarse textured soils while 29% occurred in moderately fine textured soil. In the PCA of the soil chemistry, axis 1 explained 95% of the variation, while axis 2 explained 2%. The sites were differentiated along a gradient of macronutrients dominated by the presence of calcium. However, the PCA revealed no distinction between soil samples taken from within the *E. brachycera* population and those from outside the population.

While both leafy spurge and *E. brachycera* occupy a wide variety of habitats, flea beetles have a more restricted ecological tolerance. In their native habitat, Nowierski et al. (2002) reported *A. lacertosa* to be more common on finer textured, mesic to hydric soils while *A. nigriscutis* dominated coarser, drier soils. Lym (1998) and Jacobs et al. (2001) found that introduced populations of *A. nigriscutis* in the United States did not establish on sites with greater than 80% sand. Lym (1998) reported similar results for *A. lacertosa*. Parker (2002) released flea beetles (*A. lacertosa* and *A. nigriscutis*) in Montana and South Dakota. On release sites where soil texture was documented, the average sand content was 52% (range 19%–85%). By 2003, *A. lacertosa* establishment was successful on all but one site, along the Moreau River floodplain with mesic soils in South Dakota, while *A. nigriscutis* were infrequent constituents on all sites (Murphy 2004). In the present study, soil texture fell within the range found to be acceptable for beetles; thus, soil is not considered to be a factor in preventing establishment of beetles in *E. brachycera* habitats.

In addition to soil requirements, flea beetles require the extensive leafy spurge root system to complete their life cycle, as it serves as a food source during the larval stage (Hansen et al. 1997). The difference in root systems is likely the most significant difference between *E. brachycera* and leafy spurge, particularly in terms of the ability of flea beetles to successfully complete a life cycle. Additionally, flea beetles are gregarious in nature and the dense stands of leafy spurge provide enough food for flea beetles to perpetuate, whereas the sparseness of *E. brachycera* would likely not be able to support a population of flea beetles through multiple life cycles.

The secondary objective of this study was to evaluate the potential use of *E. brachycera* by biocontrol agents in a field setting. This was meant to serve as a nochoice feeding trial in the environment in which *E. brachycera* naturally occurred. Large

Table 2. Mean percent foliar cover (±SD) for species of importance and their contribution to (dis)similarity for each study site. Percent foliar cover is mean of all microplots in each group separated into *Euphorbia brachycera* absent and present.

	Contribution to (dis)similarity (%)	Group 1 *E. brachycera* absent	Group 1 *E. brachycera* present	Group 2 *E. brachycera* absent	Group 2 *E. brachycera* present	Group 3 *E. brachycera* absent	Group 3 *E. brachycera* present	Group 4 *E. brachycera* absent	Group 4 *E. brachycera* present
Wind Cave 1	—	n^1 = 32	n = 14	n = 19	n = 7	n = 24	n = 3	n = 20	n = 2
Bare ground	32	49.4 (16.2)	50.3 (15.9)	7.3 (6.4)	21.9 (11.0)	9.0 (6.6)	15.5 (0)	3.6 (4.2)	15.5 (0)
Little bluestem	17	27.9 (14.9)	16.8 (15.3)	31.4 (14.5)	33.0 (13.4)	43.2 (10.4)	47.1 (27.4)	17.1 (13.7)	7.8 (11.0)
Big bluestem	9	2.4 (5.1)	2.8 (5.5)	16.3 (22.6)	22.9 (15.1)	7.8 (7.9)	5.2 (8.9)	T	—
E. brachycera	1	—	2.3 (0.7)	—	2.5 (0)	—	2.5 (0)	—	2.5 (0)
Wind Cave 2	—	n = 20	n = 18	n = 33	n = 4	n = 13	n = 2	n = 21	n = 0
Bare ground	39	55.1 (17.6)	55.6 (18.1)	8.8 (6.6)	5.8 (6.5)	9.5 (6.7)	9.0 (9.2)	6.8 (6.2)	—
Little bluestem	30	22.6 (13.7)	17.5 (12.6)	34.9 (8.8)	38.0 (0)	13.8 (9.7)	9.0 (9.2)	70.5 (10.9)	—
Big bluestem	8	2.3 (8.5)	1.0 (3.7)	8.8 (18.1)	3.9 (7.8)	3.8 (6.7)	—	1.1 (3.4)	—
E. brachycera	4	—	4.0 (4.2)	—	5.8 (6.5)	—	2.5 (0)	—	—
Black Hills 1	—	n = 43	n = 16	n = 26	n = 8	n = 13	n = 5	n = 12	n = 0
Bare ground	51	84.1 (12.7)	81.1 (12.8)	58.4 (13.5)	63.0 (0)	23.2 (12.7)	33.5 (10.0)	11.2 (6.4)	—
Little bluestem	10	11.8 (15.6)	16.3 (14.6)	4.3 (7.0)	7.6 (8.3)	10.7 (11.0)	9.8 (7.9)	3.0 (5.9)	—
Threadleaf sedge	8	2.3 (5.0)	2.1 (5.3)	3.5 (6.1)	6.4 (7.6)	12.0 (10.2)	12.4 (6.9)	32.4 (10.2)	—
E. brachycera	2	—	3.3 (3.3)	—	2.5 (0)	—	2.5 (0)	—	—
Black Hills 2	—	n = 26	n = 12	n = 30	n = 6	n = 19	n = 2	n = 23	n = 0
Bare ground	34	48.6 (12.6)	63.0 (0)	87.1 (4.3)	85.5 (0)	22.7 (16.8)	50.5 (17.7)	15.3 (13.6)	—
Blue grama	15	5.1 (7.1)	1.5 (4.5)	T	—	50.9 (16.8)	38.0 (0)	5.2 (7.1)	—
Ponderosa pine[2]	8	T	3.6 (10.9)	T	—	3.3 (14.5)	—	12.4 (30.9)	—
E. brachycera	3	—	4.7 (5.1)	—	6.8 (6.7)	—	2.5 (0)	—	—
Lander 1	—	n = 6	n = 12	n = 12	n = 9	n = 34	n = 8	n = 37	n = 2
Bare ground	27	38.0 (0)	38.0 (0)	38.0 (0)	38.0 (0)	26.4 (23.8)	51.1 (22.0)	16.1 (13.9)	15.5 (0)
Antelope bitterbrush	12	—	—	—	—	T	—	12.4 (19.5)	7.8 (11.0)
Skunkbrush	12	—	—	—	—	—	—	6.7 (21.8)	—
E. brachycera	1	—	2.5 (0)	—	2.5 (0)	—	2.5 (0)	—	2.5 (0)
Lander 2	—	n = 22	n = 15	n = 46	n = 10	n = 4	n = 7	n = 20	n = 1
Bare ground	40	87.8 (4.9)	86.3 (3.2)	59.4 (19.0)	67.5 (9.5)	85.5 (0)	85.5 (0)	85.5 (0)	85.5
Big sagebrush	23	T	T	17.6 (17.3)	5.2 (7.2)	T	—	1.1 (1.3)	—
Threadleaf sedge	6	T	T	5.4 (9.4)	4.1 (6.1)	T	T	3.5 (6.2)	2.5
E. brachycera	2	—	2.5 (0)	—	2.5 (0)	—	2.5 (0)	—	2.5
Lander 3	—	n = 22	n = 26	n = 25	n = 3	n = 27	n = 1	n = 21	n = 0
Bare ground	45	77.0 (16.9)	75.6 (12.1)	72.0 (11.3)	63.0 (0)	34.7 (8.1)	38.0	34.8 (24.8)	—
Antelope bitterbrush	18	—	—	T	—	1.7 (5.0)	—	30.1 (25.9)	—
Big sagebrush	16	1.6 (3.3)	T	12.6 (8.2)	11.2 (7.5)	24.1 (14.5)	—	26.4 (14.4)	—
E. brachycera	1	—	2.5 (0)	—	2.5 (0)	—	2.5 (0)	—	—
Lander 4	—	n = 42	n = 17	n = 25	n = 7	n = 14	n = 4	n = 16	n = 0
Bare ground	46	74.0 (29.0)	85.5 (0)	35.9 (25.6)	59.4 (9.4)	38.0 (0)	38.0 (0)	41.6 (17.2)	—
Antelope bitterbrush	24	13.7 (29.1)	—	24.0 (17.9)	7.6 (14.6)	5.5 (7.7)	3.9 (7.8)	6.8 (7.9)	—
Big sagebrush	12	12.0 (10.8)	1.0 (1.3)	12.2 (12.1)	2.9 (5.7)	11.3 (7.0)	8.4 (8.3)	41.0 (11.9)	—
E. brachycera	2	—	3.3 (4.4)	—	2.5 (0)	—	2.5 (0)	—	—

[1]*n* indicates number of microplots; T, trace; and —, species is absent.
[2]Includes Ponderosa pine saplings and trees up to 3 m in height; excludes overstory trees.

populations of *E. brachycera* simply did not exist at sites also inhabited by leafy spurge, so there were limits to what could be used as controls. From all 3 sites with flea beetles present, a total of 94 plants were collected and used in the greenhouse experiment that included 64 *E. brachycera* plants and 30 leafy spurge plants. Four beetles, 3 *A. lacertosa*, and 1 *A. nigriscutis* emerged from the leafy spurge plants collected from LAN1.

One site in LAN1 was adjacent to a stand of leafy spurge that had been successfully reduced by a population of *A. nigriscutis* released by the Fremont County Weed and Pest

Table 3. Percentage of soil samples in each soil texture class occurring at the study sites and percent of soil samples taken from each soil texture class inside and outside of the *Euphorbia brachycera* populations. Texture system based on United States Department of Agriculture Classification System.[1]

Soil texture	Textural class	Wind Cave sites 1–2 $n = 20$	Black Hills sites 1–2 $n = 20$	Lander sites 1–4 $n = 32$	Inside *E. brachycera* population	Outside *E. brachycera* population
					----------------at all sites ----------------	
Clay	fine	—	—	3%	—	3%
Clay loam	moderately fine	40%	15%	28%	26%	29%
Silty clay loam	moderately fine	30%	—	—	—	18%
Sandy clay loam	moderately fine	—	—	6%	3%	3%
Silt loam	medium	10%	75%	3%	34%	15%
Loam	medium	20%	10%	53%	34%	29%
Sandy loam	moderately coarse	—	—	6%	3%	3%

[1] *n* indicates number of soil samples; —, soil texture is absent.

Department (L. Baker, personal communication, 2003). Leafy spurge was declining on this site as a result of beetles, and therefore was considered to be a site known to be able to support an established beetle population. The few beetles that emerged from the leafy spurge samples indicated beetles were still present at the site and, under conditions of declining leafy spurge, may have needed alternative hosts. Baker et al. (2004) described *E. brachycera* mortality and adult feeding damage in years when leafy spurge was more abundant; however, they were unable to confirm beetle use as the cause of *E. brachycera* mortality. Although there was adult feeding damage on *E. brachycera* by *A. nigriscutis*, the damage was not severe enough to reduce the *E. brachycera* population. Indeed, the number of *E. brachycera* plants recorded at the site increased from 31 to 542 (on a 1.5-ha site) during the 3-year evaluation period of the study (Baker et al. 2004). In the present study, conducted during the growing seasons following Baker et al. (2004), there was no observed feeding damage to *E. brachycera*, nor did any beetles emerge from the collected *E. brachycera* plants.

On sites where beetles were released (BH1 and BH2), no insects emerged from any of the collected *E. brachycera* plants, nor was there any observed feeding damage on plants at either of the sites. There were no differences ($P > 0.05$) in plant mortality between collected plants and those left in the field, nor between release and nonrelease plants. From all the *E. brachycera* plants collected, the above- and belowground biomass measurements were similar for release and nonrelease plants ($P > 0.05$ for all tests). The end result was that none of the *E. brachycera* populations were adversely impacted by the presence of flea beetles.

MANAGEMENT IMPLICATIONS

The close taxonomic and geographic relationship between leafy spurge and *E. brachycera* increases the opportunity for biocontrol agents to find the native, nontarget spurge an acceptable host. Prerelease laboratory tests indicated that flea beetles have a narrow host breadth; however, not every aspect of the natural environment can be mimicked. The approach of this study was to use the ecological characteristics of two taxonomically related plants to assess the potential impact of biological control agents on a nontarget species.

Populations of *E. brachycera* occupied sites with soils and resident vegetation similar to sites invaded by leafy spurge. Unlike leafy spurge, *E. brachycera* occurs infrequently and in low densities, and did not form monotypic stands due to differences in root structure. These are fundamental differences with respect to the flea beetles' requirements to complete a life cycle. Additionally, releasing beetles on *E. brachycera* plants at locations where no leafy spurge was present created a field-based nochoice feeding trial. Lack of establishment of beetles and no evidence of an adverse impact on selected plants suggests that *E. brachycera* may be an unacceptable source of food and a poor substitute for larval development.

We know of no other studies that employed the use of ecological differences to evaluate the potential for nontarget impacts. This study provided information beyond whether flea beetles would consume or lay eggs on species taxonomically related to the target. It additionally assessed the likelihood of host-shifting by evaluating the functional differences among biocontrol agent, its intended target, and a potential nontarget species. When used in conjunction with laboratory tests, an analysis of the ecological separation of target and nontarget species will help provide a more complete evaluation of possible biocontrol agents prior to release.

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