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Temporal dynamics of leafy spurge (*Euphorbia esula*) and two species of flea beetles (*Aphthona* spp.) used as biological control agents

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Abstract

The goal of this study was to evaluate the biological control program of leafy spurge (*Euphorbia esula*) in a large natural area, Theodore Roosevelt National Park, western North Dakota, USA. *Aphthona lacertosa* and *Aphthona nigriscutis* have been released at more than 1800 points in the 18,600-ha South Unit of the park beginning in 1989; most releases have occurred since 1994. We established permanent vegetation plots throughout the infested area of the park and determined stem counts and biomass of leafy spurge and abundance of the two flea beetle species at these plots each year from 1999 to 2001. Both biomass and stem counts declined over the 3 years of the study. Both species of flea beetle are well established within the park and have expanded into areas where they were not released. *A. nigriscutis* was more abundant than *A. lacertosa* in the grassland areas we surveyed, but in all other habitats abundances were similar. Using structural equation models, only *A. lacertosa* could be shown to have a significant effect on counts of mature stems of leafy spurge. *A. nigriscutis* numbers were positively correlated with stem counts of mature stems. Previous year's stem counts had the greatest influence on change in stem counts over each 2-year time step examined with structural equation models.
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Keywords: *Aphthona lacertosa*; *Aphthona nigriscutis*; *Euphorbia esula*; Mixed-grass prairie; Natural area; Biological control; Structural equation modeling

1. Introduction

Leafy spurge (*Euphorbia esula* L.; Euphorbiaceae) is a noxious weed accidentally introduced from Eurasia into North America in the late 1800s—early 1900s via multiple shipments of contaminated crop seed (Dunn, 1985). It has spread extensively throughout pasture, rangeland, and natural areas in the Great Plains, inflicting substantial economic and ecological damage (Bangsund et al., 1999; Belcher and Wilson, 1989; Trammell and Butler, 1995). Although herbicides can be used to effectively control leafy spurge, the need for repeated applications and the tendency for infestations to occur in inaccessible areas and near water have limited their use (Bangsund et al., 1996; Kirby et al., 2000). As a result, considerable emphasis has been placed on the use of biological control organisms. As of 1996, nine insect species had been introduced into the United States for the control of leafy spurge (Hansen et al., 1997). Among the earliest of these introductions in the northern Great Plains were the flea beetles (*Aphthona* spp.; Coleoptera: Chrysomelidae).

With one exception (*Aphthona abdominalis* Duftschmid), *Aphthona* spp. introduced for the control of leafy spurge are univoltine and overwinter as larvae. Adults feed on leafy spurge leaves and flowers and may completely defoliate the plants when flea beetle densities are high (D. Larson, personal observation). The primary damage, however, is generally inflicted by the larvae, which feed on roots and thereby disrupt the flow of nutrients and provide entry points for fungal infection. Evaluations of leafy spurge abundance following

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introduction of *Aphthona nigriscutis* Foudras and *Aphthona lacertosa* Rosenhauer have revealed rapid and significant declines in leafy spurge near the point of beetle release (Kirby et al., 2000; Lym and Nelson, 2000).

Habitat affinities of the flea beetle species have been determined in their native ranges in Europe and Asia (Gassmann et al., 1996; Nowierski et al., 2002) and have guided releases of *A. nigriscutis* and *A. lacertosa* in North America. *A. lacertosa* is generally considered to be adapted to most habitats infested by leafy spurge in the northern Great Plains, with the exception of excessively sandy sites; the species is thought to be particularly well suited to more mesic sites. *A. nigriscutis* is thought to favor drier, more open habitat, and to tolerate higher concentrations of sand in the soil.

The goal of this study was to evaluate the biological control program of a large natural area, the South Unit of Theodore Roosevelt National Park, several years after flea beetles had been introduced within the park. We specifically ask: (1) is leafy spurge abundance declining over time, (2) have flea beetles expanded beyond their initial points of introduction, (3) do the two species, *A. nigriscutis* and *A. lacertosa*, favor the habitat types predicted by those favored in their native range, and (4) is there evidence that either species influences the stem density of leafy spurge?

2. Materials and methods

2.1. Study site

The South Unit of Theodore Roosevelt National Park, an 18,600-ha reserve situated along the Little Missouri River in western North Dakota, USA, is home to a relatively recent and severe infestation of leafy spurge. A 13-ha infestation of the weed, noted in 1970, had grown to some 1600 ha by 2001 (as estimated by aerial photography; S. Hagar, Theodore Roosevelt National Park, personal communication), despite an aggressive herbicide and biocontrol program begun in the 1980s, and accelerated through the 1990s. More than 1800 releases and redistributions of *A. nigriscutis* and *A. lacertosa*, averaging >2000 insects each, were made by park personnel throughout the infested areas of the park between 1994 and 1997; the earliest, smaller releases began in 1989. Each release point was recorded using a global positioning system unit and entered into a geographic information system database, although no data were collected on density or biomass of leafy spurge at the release points at that time.

2.2. Vegetation measurements

In 1999, we established $3\,m \times 5\,m$ permanently marked vegetation plots at randomly selected release points within each of six mapped vegetation types. Plant species common to these vegetation types are listed in Table 1. Two of the vegetation types, little bluestem and western wheatgrass–green needlegrass, are dominated by grasses and lack substantial woody vegetation. Little bluestem is a warm-season dominated community that favors sandier soils; western wheatgrass–green needlegrass is dominated by cool season species and occurs on more fertile soils than little bluestem. Cottonwood-Rocky Mountain juniper vegetation has a well-developed woody canopy and occupies relatively moist areas adjacent to watercourses. River bottoms have a sparse overstory of willows and harbors species tolerant of disturbance caused by frequent flooding. Hardwood draws have an overstory of green ash trees and a substantial shrub component; they occur in drainages and the relatively higher moisture availability results in high productivity. Dwarf sagebrush vegetation is dominated by large shrubs in a matrix of cool-season grasses; many of these areas occupy the lower terraces of rivers and have groundwater near the surface.

In 1999 we attempted to establish equal numbers of plots in each vegetation type at a minimum of 20 points

Table 1
The six vegetation types in which we sampled at Theodore Roosevelt National Park

Vegetation type	Most common species
Cottonwood-Rocky Mountain juniper	*Symphoricarpos occidentalis* Hook., *Pascopyrum smithii* Rydb., *Poa pratensis* L., *Calamovilfa longifolia* (Hook.) Scribn., *Melilotus officinalis* Lam., *Euphorbia esula*
Dwarf sagebrush	*Pascopyrum smithii*, *Artemisia cana* Pursh, *Symphoricarpos occidentalis*, *Stipa viridula* Trin., *Melilotus officinalis* Lam., *Poa pratensis*
Hardwood draw	*Symphoricarpos occidentalis*, *Galium boreale* L., *Prunus virginiana* L., *Poa pratensis*, *Smilacina stellata* (L.) Desf., *Achillea millefolium* L.
Little bluestem	*Schizachyrium scoparium* Michx., *Carex filifolia* Nutt., *Calamovilfa longifolia*, *Muhlenbergia cuspidatae*, *Echinacea angustifolia* D.C., *Artemisia frigida* Willd.
River bottom	*Xanthium strumarium* L., *Euphorbia esula*, *Glycyrrhiza lepidota* Pursh, *Pascopyrum smithii*, *Salix exigua* Nutt., *Elymus canadensis* L.
Western wheatgrass–green needlegrass	*Pascopyrum smithii*, *Stipa viridula*, *Bouteloua gracilis* (Kunth) Lag. Ex Griffihs, *Stipa comata* Trin. and Rupr., *Artemisia frigida*, *Carex filifolia*

Vegetation types are described in terms of the six most commonly encountered plant species in each (Larson et al., 2001).

Table 2
Number of vegetation plots sampled each year in each vegetation type

Species released	Year	CRMJ	DS	HD	LB	RB	WWGN	Total
A. lacertosa	1999	22	27	16	16	21	20	122
	2000	17	10	16	9	16	5	73
	2001	17	10	16	9	16	5	73
A. nigriscutis	1999	19	25	0	17	0	23	84
	2000	11	7	0	6	0	12	36
	2001	11	7	0	6	0	12	36
None	1999	21	25	17	16	20	18	117
	2000	17	5	17	4	12	5	60
	2001	17	5	17	4	12	5	60

Vegetation types are described in Table 1. CRMJ, cottonwood-Rocky Mountain juniper; DS, dwarf sagebrush; HD, hardwood draw; LB, little bluestem; RB, river bottom; WWGN, western wheatgrass–green needlegrass.

where *A. lacertosa* and 20 points where *A. nigriscutis* had been released as well as at 20 randomly chosen points where no release had been made. In some vegetation types it was not possible to find 20 points at which *A. nigriscutis* or *A. lacertosa* had been released, so we established as many plots as possible. In subsequent years, due to funding cuts, the number of plots was reduced. We scaled the reduced number of plots to the infested area of the vegetation type and the number of releases of each species in that vegetation type; plots were deleted at random to achieve the new sample size. Sample sizes are reported in Table 2. We divided each permanent plot into two hundred and forty $0.25\,m \times 0.25\,m$ quadrats. We counted leafy spurge stems twice each year in May and September (except in September 1999) on six randomly selected quadrats within each permanent plot. No attempt was made to determine belowground connection among stems. We distinguished seedlings (by the presence of opposite leaves near the base of the plant), flowering stems, and vegetative stems in the counts. Biomass was clipped at ground level on three randomly selected quadrats each year in July and August. We sorted biomass into eight categories (litter, leafy spurge, C_3 exotic grass, C_3 native grass, C_4 native grass, native forbs, exotic forbs, and sedges; there were no exotic sedges or C_4 grasses at our sites), dried, and determined dry mass. In this paper, we report only on biomass of leafy spurge. Quadrats used for biomass sampling were excluded from any further sampling events.

2.3. Insect sampling

We estimated adult flea beetle abundance at each of the vegetation plots, described above. Adult flea beetles were sampled using 38-cm-diameter sweep nets during peak emergence (approximately 20 June–15 July) each year. Sampling was done only when vegetation was dry, temperatures were >20 °C, and wind speed was <32 km/h. Because the sweep nets would damage the vegetation, insects were sampled around the perimeter of each vegetation plot. Each 5-m side of the plot accommodated seven sweeps and each 3-m side four sweeps, for a total of 22 sweeps/plot. The number of flea beetles of each species was summed over the 22 sweeps of the plot perimeter as an indication of flea beetle abundance at each plot. Plots were swept only once each season. We visually estimated cover of leafy spurge within the plot and within the area swept and the two were highly correlated (data not presented).

2.4. Statistical analysis

We used analysis of variance with type III sums of squares (PROC GLM in SAS; SAS Institute, 1999) to test for differences in stem counts and biomass among years (1999, 2000, and 2001) and for differences in abundance of flea beetle species among release categories (*A. lacertosa*, *A. nigriscutis*, or no release) and vegetation types (cottonwood-Rocky Mountain juniper, dwarf sagebrush, hardwood draw, little bluestem, river bottom, and western wheatgrass–green needlegrass).

Structural equation modeling (McCune and Grace, 2002, Chapter 30) was used to evaluate multivariate relationships in the data. The model presented in Fig. 1

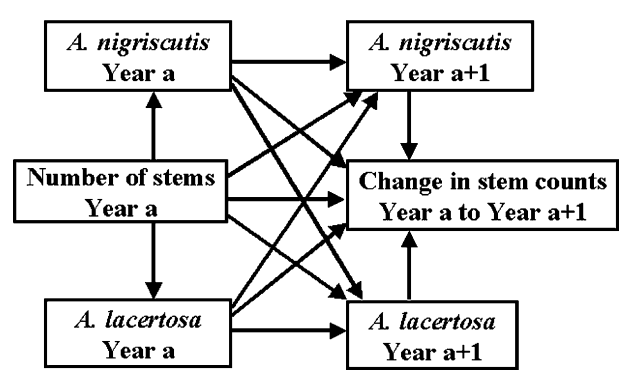

Fig. 1. Initial hypothesized model. Variables shown in boxes were measured in the field, so all are manifest variables. Arrows represent hypothesized causal relationships between the variable at the origin and the variable at the point of the arrow. The model was evaluated separately for two time periods: 1999–2000 and 2000–2001.

was used to evaluate changes in spurge stem density (i.e., number of spurge stems in year $t+1$ – number of stems in year t), *A. nigriscutis* abundance, and *A. lacertosa* abundance for the two time periods of 1999–2000 and 2000–2001. The structure of the initial model was designed to permit us to address a number of questions:

(1) Do *A. nigriscutis* and *A. lacertosa* numbers depend on spurge stem density? If so, this would confirm that spurge serves as a primary resource for the flea beetle populations in this area.
(2) Are flea beetle numbers in a given year simply a product of their numbers in the previous year or are they affected by the abundance of plants (through lag resource effects) or the other flea beetle species (through competitive interactions)?
(3) Are the observed changes in stem density related to the abundance of flea beetles; e.g., are reductions in stem density greater in areas with high beetle densities?
(4) Do changes in stem counts relate to stem density? If so, is there a positive relationship, indicating increased growth in favorable habitats or a negative relationship, indicating thinning?

As described in more detail in Chapter 30 of McCune and Grace (2002), once a multivariate model was constructed, analysis proceeded through a number of steps that included data screening, evaluating the fit of data to the initial model, and model refinement/reassessment. Data screening included inspection for outliers, normality, and heteroscedasticity. One outlier was found for an exceptionally high value of *A. nigriscutis* and this observation was dropped from the analysis, leaving a sample size of 162 observations for each year. Stem densities as well as the abundances of *A. nigriscutis* and *A. lacertosa* were log-transformed to improve their statistical characteristics.

Statistical evaluation of the model in Fig. 1 was performed using the software LISREL 8.30 (Jöreskog and Sörbom, 1996). In order to avoid concerns about lack of multivariate normality, the Robust option of Satorra and Bentler (1994) was used. All results presented in the paper are based on robust χ^2s and standard errors. Because the model tested was not developed prior to data collection, we consider this to be a use of structural equations that is "Model Generating" rather than "Strictly Confirmatory" in the sense of Jöreskog and Sörbom (1996).

3. Results

3.1. Leafy spurge abundance

Abundance of leafy spurge in May, with the exception of seedling stem counts, declined significantly over the 3 years of the study (Table 3). Seedling counts declined in 2000 but rebounded in 2001 ($F = 3.76$, df = 2, 639, $P = 0.024$). Total mature stems in spring were constant between 1999 and 2000, then declined sharply in 2001 ($F = 33.17$, df = 2, 640, $P < 0.0001$); by contrast, midsummer biomass declined between 1999 and 2000, then remained constant between 2000 and 2001 ($F = 12.83$, df = 2, 450, $P < 0.0001$). The percentage of mature stems that were in flower in spring declined steadily over the 3 years ($F = 159.47$, df = 2, 586, $P < 0.0001$). Fall mature stem counts did not vary between 2000 and 2001 ($F = 0.00$, df = 1, 324, and $P = 0.96$), the only years for which we have data, however percent flowering stems in fall ($F = 14.93$, df = 1, 287, $P < 0.0001$) and number of seedlings in fall ($F = 4.78$, df = 1, 323, $P = 0.029$) increased during that period (Table 3). Leafy spurge biomass did not vary among release point categories ($F = 0.85$, df = 2, 450, $P = 0.43$), but spring and fall mature stem counts were significantly lower on plots at nonrelease points ($F = 17.54$, df = 2, 640, $P < 0.0001$; and $F = 6.89$, df = 2, 323, $P = 0.0012$ for spring and fall stems, respectively; Table 4).

3.2. Flea beetle distribution and abundance

Both *A. lacertosa* and *A. nigriscutis* occurred in areas where they had not been released, as well as at those where they had (Table 5), indicating that the populations had persisted and spread since the releases were made. Only four nonrelease plots had no flea beetles over the 3 years we sampled; all plots located at release points still had flea beetles present in at least one of the 3 sample years. *A. lacertosa* over the 3 years did not vary between plots where it had been released versus plots

Table 3
Measures of leafy spurge abundance over 3 years

Year	Spring seedlings (mean ± SE)	Spring mature stems (mean ± SE)	Spring % flowering stems (mean ± SE)	Summer biomass (g/0.0625 m^2) (mean ± SE)	Fall seedlings (mean ± SE)	Fall mature stems (mean ± SE)	Fall % flowering stems (mean ± SE)
1999	15.4 ± 1.7^a	27.1 ± 1.1^a	40.6 ± 0.012^a	4.7 ± 0.34^a	Not measured	Not measured	Not measured
2000	7.9 ± 2.4^b	26.0 ± 1.5^a	12.3 ± 0.017^b	2.6 ± 0.30^b	1.1 ± 0.23^a	15.9 ± 1.3^a	8.8 ± 1.7^a
2001	15.7 ± 2.4^a	12.1 ± 1.5^b	6.3 ± 0.018^c	2.6 ± 0.30^b	1.8 ± 0.23^b	15.8 ± 1.3^a	17.2 ± 1.7^a

Flowering stems are expressed as a percentage of mature stems. Means are least square means from PROC GLM (SAS Institute, 1999). Statistical comparisons are within columns and numbers with the same superscript do not differ significantly.

Table 4
Stem counts of leafy spurge at biological control release points

Species released	Spring stems (mean ± SE)	Fall stems (mean ± SE)
A. lacertosa	26.4 ± 1.26^{a}	19.4 ± 1.37^{a}
A. nigriscutis	27.0 ± 1.62^{a}	15.4 ± 1.89^{a}
None	16.8 ± 1.33^{b}	11.9 ± 1.49^{b}

Spring stem counts were made in 1999, 2000, and 2001; fall stem counts were made in 2000 and 2001. Means are least square means from PROC GLM (SAS Institute, 1999). Statistical comparisons are within columns and numbers with the same superscript do not differ significantly.

Table 5
Abundance of *A. lacertosa* and *A. nigriscutis* at biological control release points

Species released	*A. lacertosa* (mean ± SE)	*A. nigriscutis* (mean ± SE)
A. lacertosa	69 ± 4.9^{a}	44 ± 4.5^{a}
A. nigriscutis	61 ± 6.0^{a}	70 ± 5.6^{b}
None	15 ± 5.1^{b}	52 ± 4.7^{a}

Counts were made of adults in 1999, 2000, and 2001. Abundance was measured as total individuals collected in 22 sweeps (1 sweep/pace) around the perimeter of each plot. Least square means and their standard errors are reported. Statistical comparisons are within columns and numbers with the same superscript do not differ significantly.

Table 6
Abundance of *A. lacertosa* and *A. nigriscutis* 1999–2001

Year	*A. lacertosa* (mean ± SE)	*A. nigriscutis* (mean ± SE)
1999	31 ± 5.05^{a}	49 ± 4.45^{a}
2000	57 ± 5.05^{b}	56 ± 4.45^{a}
2001	45 ± 6.90^{a}	45 ± 6.08^{a}

Abundance was measured as total individuals collected in 22 sweeps (1 sweep/pace) around the perimeter of each plot. Least square means and their standard errors are reported. Statistical comparisons are within columns and numbers with the same superscript do not differ significantly.

where *A. nigriscutis* had been released, but was significantly lower at nonrelease points ($F = 32.4$, df $= 2, 793$, $P < 0.0001$; Table 5). In contrast, *A. nigriscutis* counts were significantly lower at *A. lacertosa* as well as nonrelease points than they were at points where they had been released ($F = 6.65$, df $= 2, 793$, $P = 0.0014$; Table 5). Overall, abundance of *A. lacertosa* varied significantly among years with a peak in 2000; abundance of *A. nigriscutis* did not vary significantly among years (Table 6).

3.3. Flea beetle habitat

There was a significant interaction between flea beetle species and vegetation type with respect to flea beetle abundance ($F = 7.89$, df $= 11, 1582$, $P < 0.0001$; Table 7). Both species were present in all six vegetation types, although *A. nigriscutis* had not been released in either river bottoms or cottonwood-Rocky Mountain juniper habitats. *A. nigriscutis* was significantly more abundant in the more open, grassland habitats (little bluestem (*Schizachyrium scoparium*) and western wheatgrass–green needlegrass (*Pascopyrum smithii–Stipa viridula*)) than was *A. lacertosa*. Despite an overall greater number of releases, *A. lacertosa* was not significantly more abundant than *A. nigriscutis* in any vegetation type.

Table 7
Abundance of *Aphthona lacertosa* and *A. nigriscutis* in six vegetation types

Vegetation type	*A. lacertosa* (mean ± SE)	*A. nigriscutis* (mean ± SE)
Cottonwood-Rocky Mountain juniper	48 ± 6.4^{ab}	34 ± 6.4^{a}
Dwarf sagebrush	73 ± 6.2^{c}	66 ± 6.2^{b}
Hardwood draw	26 ± 8.4^{d}	21 ± 8.4^{a}
Little bluestem	40 ± 7.6^{abd}	74 ± 7.6^{b}
River bottom	28 ± 8.0^{ad}	35 ± 8.0^{a}
Western wheatgrass–green needlegrass	53 ± 6.9^{b}	78 ± 6.9^{b}

Abundance was measured as total individuals collected in 22 sweeps (1 sweep/pace) around the perimeter of each plot. Least square means and their standard errors are reported. Numbers within columns that have the same superscript do not differ significantly. Abundance of the two flea beetle species differed significantly in little bluestem and western wheatgrass–green needlegrass vegetation. See Table 1 for botanical description of vegetation types.

3.4. Relationship between flea beetles and change in leafy spurge abundance over time

Structural equation models of the same form were evaluated separately for 1999–2000 and 2000–2001 (Fig. 1) as described in Section 2. Initial results indicated the need for a slight change in model structure. While the simple correlation between *A. nigriscutis* and change in stem density was negative, analysis within a multivariate framework showed that this was a spurious relationship caused by shared dependence of these two variables on the number of stems in the previous year. For this reason, the model was reformulated to replace the directional relationship between *A. nigriscutis* and change in stems with a nondirectional correlation, which was modeled as a correlated error term (Maruyama, 1998, pp. 77–78). The only additional model changes involved making implicit model relationships into explicit ones, in this case through the deletion of nonsignificant pathways that did not have a strong biological basis and the recognition of a significant negative correlation between *A. nigriscutis* and *A. lacertosa* in the year 2000. Although nonsignificant, we left the pathway from initial stem counts to *A. lacertosa* abundance in the initial year in the model, believing that this was a biologically meaningful pathway and that to delete it would

result in overfitting the model to the data. The final models had χ^2s of 4.18 (df = 5, $P = 0.52$) and 1.25 (df = 4, $P = 0.87$) for 1999–2000 and 2000–2001, respectively, indicating good fits between models and data.

Final structural equation models for 1999–2000 (Fig. 2) and 2000–2001 (Fig. 3) were similar in form but explained different amounts of variance in the primary response variable, change in leafy spurge stem counts. The 1999–2000 interval was one during which stem densities changed little and the model accounted for only 6% of the variance in change in stem counts. During the 2000–2001 interval, stem density declined substantially and the model for this time period accounted for 42% of the corresponding variance. During 1999–2000, the change in stems was related to the abundances of *A. lacertosa* in both 1999 and 2000, as well as the initial number of stems. For the interval 2000–2001, change in stems was primarily related to the initial number of stems and secondarily to *A. lacertosa*. For both intervals, changes in stems were positively (though weakly) correlated with *A. nigriscutis*.

These results show that for both time intervals, *A. nigriscutis* and *A. lacertosa* abundances in a given year were strongly related to their abundances in the previous year, as well as to the number of spurge stems in the previous year. Negative relationships between *A. nigriscutis* and *A. lacertosa* were also indicated. Abundances of these two species were negatively correlated in 2000, as reflected in both models (Figs. 2 and 3). Additionally, *A. nigriscutis* in 2000 was negatively related to *A. lacertosa* in 1999.

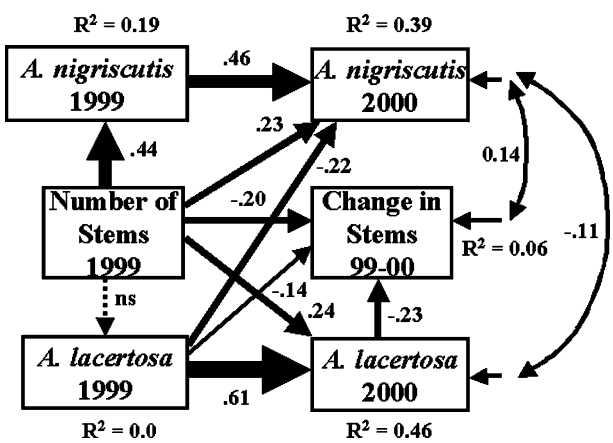

Fig. 2. Model results for 1999–2000. Single-pointed arrows indicate causal paths. Double-pointed arrows represent correlations, which were modeled as correlated errors. Path coefficients are standardized and all solid arrows indicate significant paths at $P < 0.05$; size of the arrow correlates with the magnitude of the path coefficient. The dashed arrow indicates a nonsignificant path that remains in the model. R^2 values are shown for dependent variables.

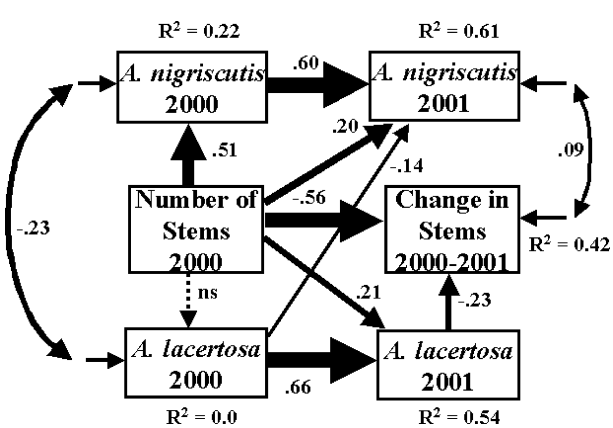

Fig. 3. Model results for 2000–2001. See description of Fig. 2.

4. Discussion

Resource managers may take the overall decline in leafy spurge abundance at Theodore Roosevelt National Park over the three years of this study as an encouraging sign. Likewise, the persistence and expansion of the flea beetle populations suggest that these biological control insects are suited to their introduced habitat. What remains to be established is the degree to which the flea beetles are responsible for observed declines in leafy spurge abundance.

4.1. Trends in abundance of leafy spurge

Populations of leafy spurge have been known to fluctuate considerably among years (Lym and Nelson, 2000), thus, trends identified in a three-year study should be viewed with caution. Nonetheless, several lines of evidence suggest that the decline we observed in leafy spurge may be related to stress imposed by flea beetles. First, there appeared to be a lag between biomass decline and stem decline, suggesting that growth waned prior to death of stems. Second, the percentage of mature stems that flowered showed steep declines, suggesting that resources were limiting to reproduction at the level of individual stems. Jacobs et al. (2001) also found a decline in flowering stems, despite consistent cover values for leafy spurge, which they attributed to a preference for flowers by *A. nigriscutis*. Finally, seedling abundance rebounded in the final year to the extent that leafy spurge biomass stayed constant, despite further decline in mature stems. This suggests that ambient resources were not limiting, since seedling establishment is strongly nitrogen-limited (McIntyre, 1972) and seedlings desiccate rapidly under dry conditions (D. Larson, personal observation). Rather, it would seem that mature stems were experiencing stress not perceived by seedlings, consistent with damage by flea beetles.

4.2. Flea beetle persistence and spread

Both *A. lacertosa* and *A. nigriscutis* seem well established at Theodore Roosevelt National Park. Consistent with observations that *A. nigriscutis* is more mobile than *A. lacertosa* (Jonsen et al., 2001), plots located at nonrelease points were more likely to harbor *A. nigriscutis*. Also consistent with reported habitat associations (Gassmann et al., 1996; Nowierski et al., 2002), *A. nigriscutis* was significantly more abundant in the two grassland habitats than was *A. lacertosa*. *A. nigriscutis* was not excluded from wetter habitats such as river bottoms, as had been suggested by associations in its native range (Gassmann et al., 1996; Nowierski et al., 2002).

4.3. Role of flea beetles in leafy spurge abundance

Results from the multivariate model found in this study are consistent with a small, but constant role for *A. lacertosa* in the observed changes in leafy spurge mature stem counts between years: as *A. lacertosa* numbers increased, the change in stems became increasingly negative. Its numbers, however, did not indicate that it closely tracked spurge stem numbers, suggesting that it was not limited by spurge abundance. *A. nigriscutis*, on the other hand, seemed to track stem density as evidenced by the fact that its numbers tended to correlate positively with leafy spurge stem counts.

At the scale of the entire leafy spurge infestation at Theodore Roosevelt National Park, both flea beetle species are most likely below their carrying capacity. Available evidence indicates that *A. lacertosa* is slower to disperse from high population areas than *A. nigriscutis* (Jonsen et al., 2001). Thus, leafy spurge may experience greater damage from *A. lacertosa* because the flea beetle tends to remain in a localized area until resources are depleted, whereas *A. nigriscutis* is more likely to disperse to other spurge patches before inflicting sufficient damage to kill stems. *A. nigriscutis* may eventually exert control of leafy spurge, if its population expands rapidly enough to overtake expansion of the spurge population (Fagan et al., 2002). We found no evidence that *A. nigriscutis* populations were increasing at the scale of our plots, however dispersal may make population increases invisible at this scale. Because we measured adults, yet damage that would kill stems is caused by larvae, the degree to which adult flea beetle counts reflect overwintering larval density will influence the modeled relationship between flea beetle numbers and change in stem counts. In the more sedentary *A. lacertosa*, adult counts likely correspond closely to larval abundance. If *A. nigriscutis* adults are more likely to disperse, however, we may be underestimating their relationship to change in leafy spurge stem counts.

In conclusion, the data presented in this paper indicate that overall population densities of leafy spurge declined during the observation period and also that they declined fastest where *A. lacertosa* was most abundant. Changes in leafy spurge density were not found to correlate with *A. nigriscutis* density during this study. Relationships in the data support the interpretation that *A. lacertosa* populations are not controlled by spurge densities while *A. nigriscutis* populations tracked spurge population densities. It is also clear that the dynamics of leafy spurge are controlled by factors other than interactions with its biological control agents. Declines in stem density were greatest where numbers of stems were highest and the multivariate analysis indicates that this effect was unrelated to influences mediated through flea beetles. Studies that look directly at larval density and damage to roots will be necessary to verify that *A. lacertosa* is the more effective control agent, as these analyses suggest.

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