ABSTRACT  
Inundative releases of beneficial insects are frequently used to suppress pest insects but not commonly attempted as a method of weed biological control because of the difficulty in obtaining the required large numbers of insects. The successful establishment of a flea beetle complex, mixed *Aphthona lacertosa* (Rosenhauer) and *Aphthona nigriscutus* Foundras (87 and 13%, respectively), for the control of leafy spurge, *Euphorbia esula* L., provided an easily collectable source of these natural enemies that enabled us to attempt inundative release as a possible leafy spurge control method in a sensitive riparian ecological zone where chemical control is restricted. Our target weed populations were small isolated patches of leafy spurge along three streams in southwestern, central, and northeastern Idaho. This study assessed leafy spurge and associated vegetation responses to inundative releases of 10 and 50 beetles per spurge flowering stem over two consecutive years. Releasing 10 beetles per flowering stem had inconclusive effects on spurge biomass, crown, stem, and seedling density. Alternatively, releasing 50 beetles per flowering stem resulted in a reduction of biomass, crown and stem density in the range of 60–80% at all three study sites, and about an ~60% reduction of seedling density at one site, compared with untreated plots. In contrast to leafy spurge, associated vegetation did not conclusively respond to beetle release, indicating that it may take more than two years for desired riparian vegetation to respond to reductions in leafy spurge competition.

KEY WORDS  
leafy spurge, *Aphthona*, biological control, riparian area, inundative release

Leafy spurge, *Euphorbia esula* L., is a perennial herb with a deep and extensive root system. The plants reproduce both vegetatively and by seed to form persistent patches that often coalesce to form large stands. Seeds are ejected and dispersed by animals, water, and human activities (Bakke 1936, Selleck et al. 1962). Established infestations of leafy spurge can dominate and displace native species, creating homogeneous plant communities (Belcher and Wilson 1989, Lym and Kirby 1987, Trammell and Butler 1995).

Leafy spurge invades a variety of land types ranging from moist flood plains to dry ridges and mountain slopes with a wide range of ecological tolerances (Selleck et al. 1962). It is found on abandoned croplands, pastures, rangeland, roadsides, and waste areas, and it often dominates bottomlands. The species will thrive on many types of soil, especially after disturbance; however, disturbance is not required for leafy spurge to colonize and displace native species (Bourchier et al. 2006).

Although riparian areas may occupy only a small percentage of the area of a watershed, they comprise a very important component of the overall landscape. A healthy, functioning riparian area provides many ecosystem services such as fish and wildlife habitat, erosion control, forage, late season stream flow, and water quality. Riparian corridors are key landscape components that provide a significant contribution to the sustainability of the surrounding ecosystem (Naiman et al. 1992).

Establishment and dominance of a nonnative invasive plant will probably have a strong detrimental impact on an ecosystem component that is extremely important to the stability of dry forest landscapes. Because leafy spurge can reproduce vegetatively and by seed production, a primary means of dispersal in riparian areas would probably occur from root segments and dislodged seeds carried downstream, especially by high water events. These rootlets and seeds become deposited in sand and gravel bars along with upstream alluvial deposits as water levels recede. The weed rapidly roots and spreads forming isolated patches of leafy spurge that exclude establishment of other vegetation. Thus, riparian areas become popu-
luted by small patches of leafy spurge, with a continual downstream dispersion (Sheley et al. 1995, Planty-Tabacchi et al. 1996).

Control of leafy spurge in riparian areas is of critical importance, yet it is difficult to achieve. Herbicide use is restricted because of concerns about their impacts on fish and aquatic organisms (Folmar et al. 1979, Buhl and Faerber 1989, Austin et al. 1991). Herbicides can be oversprayed into flowing water or leach into the hyporheic zone and enter the free-flowing stream where they may be diverted downstream to irrigate ranch or farmland. The lack of viable options to manage leafy spurge in riparian areas has hindered ranchers and other land managers in their attempt to control this nonnative invasive weed (Stohlgren et al. 1998).

The use of inundative releases of insects as a biological control is not a new concept. There are numerous examples of the use of augmented and inundative insect releases to suppress, eradicate, or control pest insects (Smith and Hubbes 1986, Grenier 1988, Purcell 1998, Myers 1998). However, there is little documentation of inundative releases of insects to provide rapid suppression and general control of a plant pest (but see Stinner 1977).

*Aphthona* spp. adults emerge from the soil in late May to early July and feed on spurge foliage. After mating, the females lay eggs near a leafy spurge crown just below the soil surface. In 8 d, the larva emerge and feed on the plant roots. Larval feeding and secondary root infection by pathogens (Caesar 1994) damage the roots resulting in a decrease in stem density and overall plant vigor. *Aphthona* overwinters as larvae in the soil and resume feeding the following spring when soil temperatures reach ≈15°C. The larvae pupate after feeding for a few weeks, and emerge as adults that feed on the succulent foliage of young leafy spurge plants.

*Aphthona* flea beetles seem amenable to inundative release strategies for several reasons: 1) they have demonstrated an ability to reduce populations of leafy spurge in xeric habitats with and without repeated releases (Lym and Nelson 2000); 2) the species may be collected in large numbers from numerous locations (Van Hezewijk and Bourcher 2005); and 3) *Aphthona* do not willingly disperse in the presence of a suitable leafy spurge population; they tend to remain at high densities within local populations of leafy spurge.

We hypothesized that inundative releases of *Aphthona* flea beetles would quickly reduce the abundance of leafy spurge in riparian areas. In addition, flea beetle populations may persist and provide continued management, at least until the next flood event. We also propose that the more beetles released, the more assured and the more rapid the reduction of leafy spurge.

**Materials and Methods**

**Plot Establishment.** In 2005, nine plots were situated >50 m apart within the riparian corridor of each of three watersheds in Idaho: in the Owyhee River watershed along Boulder Creek, east of Jordan Valley, OR (42°49’ 31” N, –116°46’ 49” E; elevation, 1,336 m); along the South Fork of the Boise River, west of Featherville, ID (43°36’ 29” N, 115°13’ 22” E; elevation, 1,385 m); and in northeast Idaho, along Beaver Creek, north of Spencer, ID (44°22’ 3” N, 112°10’ 54” E, 1,674 m). Average annual precipitation ranges from 28.5 cm (Featherville) to 33.3 cm (Spencer). All plots have had mild historical grazing and are characterized by high desert biomes with subsequent riparian communities. The soil types for all sites are similar and primarily composed of quartzite, quartz monzonite, residuum, colluvium, alluvium, andesite, and basalt. All plots were either 6 by 4 m or 8 by 3 m, depending on the dimensions of the small isolated patch of leafy spurge being targeted. Each 24-m² plot was further divided into 1-m² subplots. At each study site treatments were randomly applied: three plots were untreated (control), three received 10 *Aphthona* flea beetles per flowering leafy spurge stem, and three plots received 50 beetles per leafy spurge flowering stem. In 2005 and 2006, during the last week of June, flea beetles were released on a per square meter basis for even distribution, with the number of beetles per plot based on the number of flowering stems observed on the plot in the first study year. The plots were not entered during the 3 wk after release to allow beetles time to establish and oviposit. We assessed the ability of the flea beetles to survive and complete their life cycle in riparian areas in 2006 and 2007 by sampling the study plots by using sweep nets for flea beetle population establishment. In winter 2005, one plot at Jordan Valley (untreated control), and one plot along the South Fork of the Boise River (10 beetles per flowering stem) were washed out in high water events; they were replaced in the spring of 2006. The plots along Spencer Creek were trampled by cattle in 2007. Sweep net samples taken during the summer of the year after the first release (2006) yielded *Aphthona* beetles within some of our study plots at all three sites, even though two of the sites flooded in the spring.

*Aphthona* spp. **Flea Beetle Collection.** The necessary flea beetles [87% *Aphthona lacertosa* (Rosenhauer) and 13% *Aphthona nigricutis* Foudras] were obtained from an insectary located in central Idaho along the South Fork of the Boise River. Sweep nets were used to collect beetles during the third week of June. The beetles were measured volumetrically (10 ml is ≈1,000 flea beetles) and placed into cardboard ice cream containers with a bouquet of leafy spurge. Containers were placed in coolers with ice packs. Releases were made either the same day or the day after collection.

**Leafy Spurge Sampling.** Leafy spurge crowns, stems, and seedlings were tallied around peak standing, crop in late June. In the fall, after senescence, all leafy spurge from the central 8-m² area of plots was harvested by square meter and oven-dried at 65°C to a constant weight. We considered all stems <7.5 cm in height to be seedlings and stems within 7.5 cm of a distinct crown were considered as part of the same plant or crown.
Table 1. Average leafy spurge biomass and stem count per square meter [mean (SE)] by treatment, year, and location

<table>
<thead>
<tr>
<th>Location</th>
<th>Treatment</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaver Creek</td>
<td>Control</td>
<td>42.13 (3.98)/58.68 (4.55)</td>
<td>7.58 (1.35)/23.80 (3.77)</td>
<td>2.88 (0.80)/10.68 (1.41)</td>
</tr>
<tr>
<td></td>
<td>10 beetles</td>
<td>53.84 (10.72)/40.09 (3.31)</td>
<td>6.49 (2.06)/2.45 (0.48)</td>
<td>3.95 (1.00)/5.15 (0.79)</td>
</tr>
<tr>
<td></td>
<td>50 beetles</td>
<td>75.86 (6.78)/62.48 (5.02)</td>
<td>1.15 (0.49)/1.40 (0.58)</td>
<td>1.85 (0.31)/3.93 (0.61)</td>
</tr>
<tr>
<td>South Fork Boise River</td>
<td>Control</td>
<td>32.41 (4.21)/96.62 (6.30)</td>
<td>38.49 (5.54)/57.76 (6.37)</td>
<td>31.61 (4.64)/58.05 (6.13)</td>
</tr>
<tr>
<td></td>
<td>10 beetles</td>
<td>36.59 (7.10)/101.23 (5.49)</td>
<td>35.84 (4.52)/53.27 (3.80)</td>
<td>21.69 (4.43)/55.69 (3.51)</td>
</tr>
<tr>
<td></td>
<td>50 beetles</td>
<td>41.44 (7.89)/96.55 (9.38)</td>
<td>8.08 (1.08)/23.54 (2.58)</td>
<td>6.49 (1.38)/32.40 (4.10)</td>
</tr>
<tr>
<td>Boulder Creek</td>
<td>Control</td>
<td>49.67 (12.59)/69.0 (5.09)</td>
<td>41.33 (7.46)/45.04 (3.93)</td>
<td>35.74 (5.92)/49.43 (3.90)</td>
</tr>
<tr>
<td></td>
<td>10 beetles</td>
<td>42.47 (4.01)/63.77 (4.11)</td>
<td>25.85 (2.80)/35.76 (2.80)</td>
<td>44.08 (4.27)/65.98 (3.59)</td>
</tr>
<tr>
<td></td>
<td>50 beetles</td>
<td>49.51 (14.09)/69.0 (5.09)</td>
<td>25.87 (3.20)/44.45 (3.95)</td>
<td>13.59 (3.03)/24.19 (3.70)</td>
</tr>
</tbody>
</table>

Associated Species Sampling. The current-year cover of annual forb, perennial forb, and graminoid was estimated within the eight interior subplots to avoid edge effects and to minimize disturbance caused by sampling. A square meter polyvinyl chloride (PVC) frame was used to define subplot boundaries by placing it around pin flags that marked the subplot corners. The cover of vegetation rooted within PVC frames was estimated in each subplot, and plants hanging over the frame were included in cover values. Cover was estimated as the observed portion of the subplot covered by each category of plant from directly overhead. Thus, small spaces between branches and leaves were included in the cover value. Cover values were recorded to the nearest 5%. Differences in cover estimates among observers was minimized by calibrating estimates with one another at the start of each day or when community composition changed enough to potentially alter cover readings.

Statistical Methods. The model for leafy spurge data was as follows:

\[ y_i \sim N(\mu_{ij}, \alpha_i) + \beta_{i0} + \gamma_{i0} + \delta_{i} + \varphi_{ai} + \sigma \]

where \( y_i \) is a leafy spurge response variable and \( N(\mu, \sigma) \) is the normal distribution with mean \( \mu \), standard deviation \( \sigma \). For some response variables, small numbers of observations equaled zero and so were set equal to the smallest value in the data set. The observation index \( i \) ranged from 1 to 54 = number of plots \( \times 2\) study years. The \( \mu \) are site means with \( j(\omega) \) signifying site membership (Gelman and Hill 2007). For example, \( j(8) = 3 \) signifies the eighth observation is on site 3. The \( \alpha \) are year effects, the \( \beta \) are treatment effects, and the \( \gamma \) are treatment \( \times \) site interactions. The \( x \) vector contains the 2005 pretreatment data standardized to mean 0, standard deviation 1, so \( \delta \) accounts for pretreatment conditions. The \( \varphi \) are plot effects, which were assigned normal distributions with means 0 and standard deviations \( \tau_\varphi \) and \( \tau_\varphi \) respectively. Our hierarchical Bayesian approach to parameter estimation required assigning prior distributions to all \( \tau, \mu, \alpha, \beta \) and to \( \delta \) and \( \sigma \). These parameters were assigned uniform distributions, except the random error variance that was assigned, \( \mu(\sigma^2) = \sigma^{-2} \).

These are standard, noninformative priors (Gelman et al. 2004). We used a Gibbs sampler constructed in FORTRAN to simulate the joint posterior distribution of model parameters (Intel Corporation 2003).

The 95% Bayesian confidence intervals we present are interpreted simply as having a 0.95 probability of bracketing the parameter. The intervals estimate differences between the not-treated controls and the two beetle release treatments.

Results

During winter 2005–2006, flooding occurred on one Jordon Valley control plot and one Featherville plot that received 10 beetles per stem. Replacement plots were established in spring 2006. In the analysis, missing pretreatment values for the replacement plots were imputed as the average of 2005 values. Based on scatter plots, the replacement plot receiving 10 beetles per stem in 2006 but not 2005 responded similarly to plots receiving 10 beetles in both years, so no other adjustments were deemed necessary in the analysis. The Dubois site was unintentionally subjected to cattle grazing in 2007. Nevertheless, patterns in the 2007 Dubois data were similar to patterns observed in data from the other two sites, so we did not reject the data from the analysis. Because equation 1 contains terms for years, sites and treatment \( \times \) site interactions, the only assumption implicit in including the data are that cattle activities influenced treated and not-treated plots similarly.

Leafy Spurge. Because the confidence intervals greatly overlap zero, releasing 10 beetles per flowering stem had inconclusive, potentially small effects on leafy spurge biomass, crown, stem and seedling density (Table 1; Fig. 1). However, releasing 50 beetles conclusively reduced leafy spurge, and there is considerable evidence suggesting the reductions were quite large. For example, many of the most likely parameter estimates are between \(-1\) and \(-2\) on the natural log scale that corresponds to 63% = \(1 - e^{-1}\) and 86% = \(1 - e^{-2}\) reductions in leafy spurge, respectively, on the proportional scale (Table 1; Fig. 1). Equation 1 assumes proportional changes in leafy spurge due to treatment were consistent across years within a given site. Fitting a model that allowed treatment effects to vary by year provided inferences similar to those obtained from equation 1, so we based our conclusions...
on the simpler model. This indicates that treatment effects did not vary appreciably by year.

**Associated Vegetation.** There were no conclusive differences between treatments in cover of forbs or grasses at any of the sites (Fig. 2). However, the confidence intervals for cover variables overlap large ranges of values indicating the data were not precise enough and/or the data set was not large enough to rule out potentially large negative and positive effects.

**Discussion**

Inundative releases of *Aphthona* flea beetles at a rate of 50 beetles per flowering stem reduced leafy spurge...
biomass, and the densities of crowns, and stems at all three study sites, and seedling density at one of the three study sites. Most reductions were between 60 and 80%, based on the most likely parameter values. By facilitating the temporary establishment of high *Aphthona* densities, the vigor of leafy spurge populations within riparian areas was reduced in a short time. Of special significance is the adaptation of a protocol to use *Aphthona* flea beetles in a novel manner and in areas where traditional herbicide controls are often prohibited. *Aphthona* spp. are well suited for inundative releases because they are easily collected and reach high densities over small areas (Van Hezewijk and Bourchier

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**Fig. 2.** Most likely values (dots) and 95% Bayesian confidence intervals (bars) describing plant community responses to release of 10 or 50 *Aphthona* beetles per leafy spurge stem at three sites (Beaver Creek, Site 1; South Fork Boise River, Site 2; and Boulder Creek, Site 3). In addition to estimating natural-logged differences between treatments and controls, the confidence intervals estimate proportional differences. For example, the most likely value for forb cover in the 10 beetle treatment at Site 1 is 0.2, which corresponds to a $22\% = 1 - e^{-0.20}$ increase in forb cover.
Flea beetles have demonstrated the ability to dramatically reduce populations of leafy spurge and are relatively slow to disperse when there is an abundance of host plants available (Lym and Nelson 2000). It was uncertain how well flea beetles would survive in the moist conditions associated with a high water table in riparian areas that occasionally flood. However, sweep net samples from our plots in 2006, before release of beetle treatments frequently yielded flea beetles. We assumed these were progeny of beetles released the previous year, suggesting a limited ability of the beetles to withstand flooding events.

We detected no conclusive differences in forb and grass cover between treatments. This was somewhat surprising because leafy spurge has been shown to compete intensively with associated species under a wide range of conditions (Rinella and Shelley 2005, Rinella and Luschei 2007). The breadth of our confidence intervals that estimate grass and forb responses are quite wide (Fig. 2), indicating that our data set was not large and/or precise enough to exclude a wide range of potential effects. Also, it may be that resident plant communities require more than two growing seasons to begin recovering from competitive suppression by leafy spurge. Huffaker and Kennett (1959) reported increases in forbs and grasses after introductions of a biocontrol agent that reduced populations of Klamath weed, Hypericum perforatum L. Higher diversity of nontarget species occurred 2–6 yr after A. nigriscutus and decline in levels of leafy spurge in Manitoba grasslands (Mico and Shay 2002). Lesica and Hanna (2004) present a whole written discussion of the indirect effects of biological control on plants.

Riparian corridors possess an unusually diverse arrangement of species and environmental processes. This ecological diversity is caused by variable flood regimes, geomorphic channel processes, and other influences on the fluvial corridor (Naiman et al. 1993). Processes caused by flood disturbances play a critical role in the maintenance of biodiversity along river corridors (Hughes et al. 2005). Therefore, riparian ecosystems may be described as mobile habitat mosaics characterized by variability and unpredictability (Hughes et al. 2005). These riparian communities are susceptible to disturbance events at different spatial-temporal scales depending on proximity to the stream channel. Hence, rapid and/or frequent change in the composition of riparian vegetation is likely.

Other studies have shown similar Aphthona spp. caused reduction in the cover of leafy spurge (Table 2) over time. However, there is a lot of variation in performance among locations. Several studies show reductions in the abundance of leafy spurge at the point of release with a gradient of spurge coverage increasing with distance. Kalischuk et al. (2004) documented significant reductions of leafy spurge up to 5 m away from the release point 3 yr after release. Lym and Nelson (2000) showed that A. nigriscutus reduced the coverage of leafy spurge by 65% 16 m from the release point by 3–5 yr after release. Mico and Shay (2002) also documented significant reductions of the cover, height, density, and biomass of leafy spurge near the point of release.

Leafy spurge is well established in North America and will probably never be eradicated. At best, land managers hope to reduce the abundance and impacts of this invasive weed to levels that are ecologically and economically tolerable. This study has shown that within a 1-yr time frame, Aphthona flea beetles can appreciably reduce leafy spurge. This method has practical applications in riparian corridors or other environmentally sensitive areas containing small isolated patches of leafy spurge where herbicide use is restricted or prohibited.

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