Decline of spotted knapweed density at two sites in western Montana with large populations of the introduced root weevil, *Cyphocleonus achates* (Fahraeus)

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Abstract

Spotted knapweed is an important weed of rangeland in the northwestern United States and western Canada, and has been the focus of considerable biological control efforts. *Cyphocleonus achates*, a Eurasian root weevil, has been released as a biocontrol agent against the weed in many areas of Montana and the Pacific Northwest. Spotted knapweed plant density was monitored over a 11-year period (1993–2004) at two sites in western Montana where *C. achates* was released. Spotted knapweed density declined significantly over time at both sites (99 and 77%, respectively), after *C. achates* numbers increased dramatically at both sites. The average annual population growth rate of *C. achates* during 1993–1995 was 14% at the two sites, compared to 10% during 1995–1998. The weevil population radially expanded a distance of 73 m per year at one study site compared to 99 m per year at the other site. The estimated *C. achates* population increase at the two sites was similar: in 1998, the *C. achates* population estimate for one site was 90,776 in a 23 ha area of occupation, compared to 97,173 in a 29 ha area at the other site. Evidence suggests that *C. achates* played a major role in the spotted knapweed population decline. Following the decline of spotted knapweed, two annual exotic weeds, *Bromus tectorum* and *Descurainia sophia*, became major components of the new plant community, with *B. tectorum* becoming the dominant plant. By 2004, *B. tectorum* comprised 89 and 50% of the replacement vegetation at the two sites.

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Keywords: Spotted knapweed; *Centaurea stoebe* subsp. *micranthos*; *Centaurea maculosa*; *Centaurea biebersteinii*; *Cyphocleonus achates*; *Bromus tectorum*; Biological control; Post-release impact evaluation; Agent spread; Agent population growth

1. Introduction

Spotted knapweed, *Centaurea stoebe* L. subsp. *micranthos* (Gugler) Hayek (=*Centaurea maculosa* Lamarck (Oechsmann, 2001); =*Centaurea biebersteinii* de Candolle), is a deeply taprooted perennial plant from Eurasia that is a serious weed on rangelands of the northwestern United States. First reported in North America in 1893, the plant now infests over 3 million ha of rangeland and pasture in 14 states and two Canadian provinces (Lacey, 1989; Sheley et al., 1998). Spotted knapweed reduces livestock and wildlife forage (Thompson, 1996), increases surface water runoff and soil sedimentation (Lacey et al., 1989), and significantly lowers plant diversity (Tyser and Key, 1988).

Many control options are effective against spotted knapweed, including herbicides, mowing, grazing, tillage, and biological control (Duncan et al., 2001; Sheley et al., 1998). However, chemical and cultural practices are often very expensive, temporary, and usually limited to accessible areas.

Spotted knapweed has been the focus of considerable biocontrol effort in Montana. Twelve Eurasian insect species have been introduced into Montana against spotted knapweed. Seven species are having an impact on the plant in some areas of western Montana. These insects include a root weevil, *Cyphocleonus achates* (Fahraeus) (Coleoptera: *Chrysomelidae*; *Curculionidae*; *Cucujidae*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoidea*; *Curculionoide
Curculionidae), a root moth, *Agapeta zoegana* L. (Lepidoptera: Tortricidae), two seed head flies, *Urophora affirmis* Frauenfeld and *U. quadrifasciata* (Meigen) (Diptera: Tephritidae), a seed head moth, *Metzneria paucipunctella* Zeller (Lepidoptera: Gelechiidae), and two seed head weevils, *Larinus obtusus* Gyllenhal and *L. minutus* Gyllenhal (Coleoptera: Curculionidae). The *Urophora* spp., *Larinus* spp., and *M. paucipunctella* are causing significant reductions in spotted knapweed seed production (Story et al., 1989, 1991; Smith and Mayer, 2005). *A. zoegana* is causing reductions in spotted knapweed biomass in some areas (Story et al., 2000).

Because of its proven ability to cause mortality to spotted knapweed plants (Corn et al., in press), the weevil, *C. achates*, may be the most effective agent. The biology, host specificity, and potential impact of *C. achates* were described by Stinson et al. (1994). Larvae mine into the root cortex, where they often induce formation of a root gall. Feeding by older larvae causes considerable damage, especially to small plants or plants containing multiple larvae. The weevil overwinters as a larva in the root and emerges as an adult from mid July to October. The large (14 mm long), long lived (8–15 weeks) adults are not known to fly. The weevil has one generation per year.

We report the changes in spotted knapweed density, the response of competing vegetation, and the changes in the *C. achates* population over a 11-year period at two sites in western Montana where *C. achates* was released.

### 2. Materials and methods

The study was conducted at two sites in the Bitterroot Valley of western Montana. One of the sites was located in a 23 ha spotted knapweed-infested field on the Teller Wildlife Refuge (TWR) near Corvallis, Montana, while the other site was located in a 29 ha spotted knapweed-infested field on the Lee Metcalf National Wildlife Refuge (LMR) near Stevensville, Montana. The TWR and LMR sites are about 22 km apart. Both sites have had occasional livestock use, but have been largely unmanaged for at least 20 years. Both locations contained dense spotted knapweed infestations (ca. 40 and 14 plants/m² at TWR and LMR, respectively) at the start of the study. Soils at TWR are Dominic loose, sandy soils. The average annual precipitation is 29.7 cm, and the average frost-free period is 111 days.

A total of 325 *C. achates* were released at TWR during 1989 through 1990 while, at LMR, 694 *C. achates* were released during 1991 through 1992 (Story et al., 1997). Population counts of *C. achates* were made in September 1993, 1995, and 1998 at the TWR and LMR release sites. The counts were made by creating 15-person-minute counts (three people observing for 5 min) of adult *C. achates* in a 5 x 5 m plot. The number of plots counted per site increased from four and eight at LMR and TWR, respectively, in 1993 to 31 and 27 in 1998 as the *C. achates* population expanded throughout the study sites. Plots were located in a grid system to determine the density of the weevil and the boundaries of the *C. achates*-occupied area. Population estimates of *C. achates* in the *C. achates*-occupied portions of the spotted knapweed infestations at each site were calculated by multiplying the mean number of adult *C. achates*/m² by the area of the *C. achates*-occupied portion of the spotted knapweed infestation.

Measurements of spotted knapweed density (plants/m²) were made annually in June or July during 1993 through 1999, and in 2004 at TWR, and 1993 through 2004 at LMR using the point-centered quarter method described by Dix (1961). The distance of the nearest knapweed plant (not seedling) from a survey pin (point) in each of four quadrants around the pin was measured, for a total of four measurements per point. Ten points were sampled along each of five permanent transects, for a total of 200 measurements. Spotted knapweed density was not measured at TWR during 2001 through 2003 due to scarcity of plants. Density of all plant species was measured in June at both sites in 2002 and 2004 using the same five transects used in previous years for the knapweed-only measurements, and the Dix (1961) method. Information on all plant species was only collected in the latter years at the two sites because, prior to that time, spotted knapweed formed a near-monoculture.

Spotted knapweed density was measured at three sites without *C. achates* within 8 km of TWR and LMR, respectively (=six total sites), in early July 2005, by recording the spotted knapweed density in 10 random 50 x 50 cm plots per location. The absence of *C. achates* at these sites was confirmed by examining roots of 10 large, randomly-collected spotted knapweed plants at each site. The 10 plants were selected by collecting the nearest mature spotted knapweed plant at 3 m intervals along a line transect.

#### 2.1. Statistical analysis

Count data for *C. achates* were transformed by $\sqrt{Y + 0.5}$, while count data for spotted knapweed were transformed with $\sqrt{Y}$. Linear regression analysis was used to describe the relationship between year and spotted knapweed plant density, and year and *C. achates* density at both sites. The mean number of spotted knapweed plants/m² for each of the five transects per year was used in the regressions for each site (i.e., $n = 40$ for TWR and 50 for LMR). All regressions were conducted using JMP IN (SAS Institute, 2001).

The annual population growth rate of *C. achates* was estimated with the model:

$$Y_{t+1} = Y_t (1+r),$$

where $x$ is year and $Y_{(x+1)}$ is the population in the year following $Y_{(x)}$. Growth rate ($r$) was calculated iteratively to fit the data (Renshaw, 1991). Rate of range expansion
of *C. achates* was determined by regressing the square root of the area occupied by *C. achates* against time (Shigesada and Kawasaki, 1997).

### 3. Results and discussion

Spotted knapweed density declined significantly over the 11 years at both sites, but the change was most dramatic at TWR (Fig. 1). Spotted knapweed density declined by 99% at TWR, compared to 77% at LMR. Spotted knapweed density declined sharply at TWR during 1993 through 1999, but after 1999 the density leveled out at about 0.4 knapweed plants/m² (F₁,₈ = 7.2, P = 0.03, r² = 0.74). At LMR, spotted knapweed density at 4.8 plants/m² was still in decline in 2004 (F₁,₈ = 5.7, P = 0.04, r² = 0.42) but, based upon the regression, the rate of decline (~0.8 spotted knapweed plants/m²/year) should not be sustained for more than an additional 5 years.

Changes in the mean number of *C. achates*/m² at each site are shown in Table 1 and Fig. 1. The mean number of *C. achates*/m² during the first sampling year (1993) was lower at TWR (0.06) than at LMR (0.69), but the area of *C. achates* occupation at TWR was larger, resulting in a larger population estimate at TWR. In 1995, the mean number of *C. achates*/m² and the area of *C. achates* occupation was similar at both sites. By 1998, the *C. achates* density/m² had increased dramatically (0.53 at TWR and 0.43 at LMR) across large areas. This increase in *C. achates* density and increase in occupied area over the five years was reflected in dramatic increases in estimated total populations at both sites. The increase of the *C. achates* estimated population from 1993 to 1998 at both sites was highly significant (TWR: F₁,₉₅ = 58.0, P < 0.0001; r² = 0.38; LMR: F₁,₈₆ = 62.8, P < 0.0001, r² = 0.42). An exponential growth model showed that the average annual population growth rate of *C. achates* during 1993–1995 was 12 and 15% at TWR and LMR, respectively, compared to 8 and 12% during 1995–1998. The reduced growth rate in the later years was likely due to increased dispersion of the population. A model for estimating the rate of *C. achates* range expansion indicated that the weevil population radially expanded a distance of 73 m per year at TWR compared to 99 m per year at LMR. The estimated *C. achates* population increase at the two sites was similar: In 1998, the population estimate for TWR was about 91,000 *C. achates* in a 23 ha area of occupation, and about 97,000 in a 29 ha area of occupation at LMR. Very high numbers of adults were observed on vertical structures (plants, fence posts, etc.) throughout both sites in 1998, which corroborated our high population estimates at the two sites. Casual observations after 1998 indicated the *C. achates* total population declined as the spotted knapweed population was reduced.

The low number of sample years for the *C. achates* populations (three) did not provide for a robust regression of *C. achates* populations against spotted knapweed population densities. Additionally, management policies at both sites prevented the use of insecticidal controls, and the use of exclosure cages was ineffective, so a more rigorous examination of the cause and effect relationship between the spotted knapweed and *C. achates* densities was not possible. However, the association between the increase of *C. achates* and the decrease of spotted knapweed over time was strong, especially at TWR (Fig. 1). Spotted knapweed populations remained high in 2005 (in fact, higher than initially observed at the study sites) at the six locations near the study areas that were not occupied by *C. achates* (Table 2), providing corroborating evidence that *C. achates* had an effect on spotted knapweed density at the study sites. As mentioned earlier, a controlled study has shown that *C. achates* can cause significant mortality to spotted knapweed (Corn et al., in press).

The fact that the spotted knapweed decline was evidently due primarily to one agent, *C. achates*, is contrary to the long-held belief that successful biocontrol of spotted knapweed would require the introduction of numerous host-specific agents (Harris and Cranston, 1979). Six other biocontrol agents (*A. zoegana, U. affinis, U. quadrifasciata, M. paucipunctella, L. obtusus, and L. minutus*) were...
established in the valley area containing the two study sites and the six sites without *C. achates* during the study. Because spotted knapweed was still abundant at the six sites without *C. achates*, the combined effect of the other six agents was apparently not enough to reduce spotted knapweed density. However, the possible complementary effects of the other six agents cannot be discounted without further study. For example, the significant seed reduction being caused by the seed head agents may prevent a resurgence of the spotted knapweed at the two study sites now that the *C. achates* population is greatly reduced. Also, the other six agents may be more effective in other areas within the estimated 1.6 million ha of spotted knapweed in Montana.

Story et al. (2000) reported that the root moth, *A. zoegana*, can cause significant reduction in spotted knapweed above-ground biomass and number of capitula per plant, but this agent has not demonstrated any obvious effect on spotted knapweed density. Because *A. zoegana* disperses much faster than *C. achates*, the moth has been well established in the area much longer than *C. achates*. In view of the lesser impact of *A. zoegana* on spotted knapweed despite being well established for a longer period, we conclude that *C. achates* is a much more effective agent than *A. zoegana*. Grass competition did not complement the impact of *A. zoegana* (Story et al., 2000), but because *C. achates* causes mortality to spotted knapweed plants, grass competition may have a significant complementary effect to *C. achates* attack.

Below average precipitation was experienced in western Montana in 1995 and during 2001–2002 (Fig. 2, NOAA http://cdo.ncdc.noaa.gov/ancsum/ACS?stnid=20012392). Since precipitation was at or above normal during the years that the *C. achates* population significantly increased, drought was probably not a prominent factor in the decline of spotted knapweed. Precipitation was also not a factor in the differing spotted knapweed populations between the two study sites and the six sites without *C. achates* because the eight sites were all within a 29 km-wide area so precipitation was similar at all sites. However, spotted knapweed, which is a relatively drought-tolerant plant, may become more vulnerable to dry conditions when under heavy attack by *C. achates*.

Spotted knapweed comprised only about 2 and 10% of total vegetation at TWR and LMR, respectively, in 2002 (Table 3). In 2002, two perennial grass species, *Agropyron caninum* (L.) Beauv. and *Poa pratensis* L., and an annual weedy forb, *Descurainia sophia* (L.) Webb, were major components of the plant community at TWR in 2002, while *A. caninum*, *D. sophia*, *P. pratensis*, and *Bromus tectorum* L. were dominant plants at LMR. Weedy, exotic annual species comprised 65 and 50% of the vegetation at TWR and LMR, respectively, in 2002. In 2004, the biggest change was the dramatic increase of *B. tectorum* at both sites. That plant comprised 89% of the vegetation at TWR and 50% at LMR. The absolute density of those

![Fig. 2. Annual precipitation in the area encompassing the two study sites and the six sites without C. achates. The dashed line represents the long term average annual precipitation (29.7 cm).](image-url)
species with less than 30 individuals for the total sample was not presented in Table 3 because measurements of species with fewer than 30 individuals are not accurate when using this sampling method (Dix, 1961).

Reasons for the marked increase in *B. tectorum* were not determined, but may have been related to the dry springs of 2001, 2002, and 2004 (Fig. 2). Due to its early spring growth habit, *B. tectorum* is able to outcompete other plant species, especially during dry years (Thill et al., 1984; Upadhyaya et al., 1986). Although *B. tectorum* provides good early spring forage, it greatly increases the incidence of range fires in the summer (Upadhyaya et al., 1986), so its replacement of spotted knapweed may not be perceived as an improvement by land managers.

Initially, we hypothesized that, because herbicides and mechanical weed control methods were not used at these sites, a decline in spotted knapweed density would result in a noticeable increase in the density of perennial grasses and forbs. However, that did not happen, at least in the first few years following the decline in spotted knapweed density. Instead, the spotted knapweed was generally replaced by weedy annual grasses and forbs. A study on the effects of biocontrol of leafy spurge (*Euphorbia esula* L.) in Montana revealed that biocontrol slowed the loss of plant diversity at sites with no herbicide history. In addition, biocontrol introduction was not associated with a disproportionate increase in other invasive exotic species (Lesica and Hanna, 2004). The fact that the spotted knapweed decline at our study sites was followed by an increase in other invasive exotic species was apparently due to an absence of perennial plant species. The absence was possibly due to the depletion of the seed bank of those plants after the long domination by spotted knapweed.

Our study was not long enough to determine the eventual plant community composition following the decline of spotted knapweed. It is possible that the current expansion by *B. tectorum* and other weedy annuals is simply a reflection of the normal successional progression at those sites following the spotted knapweed decline. But, *B. tectorum* may remain the dominant plant at the study sites for many years due to the plant’s highly competitive nature and the paucity of perennial plants. The replacement of spotted knapweed by other weed species suggests that, regardless of the control method used, previously-infested landscapes will not necessarily return to the original plant community immediately unless land management strategies are altered.

The apparent impact of *C. achates* on spotted knapweed in this study provides considerable hope for the potential of biological control against this weed in Montana and the Pacific Northwest. But, considerable work is needed before large-scale reductions of the weed can be expected. Because *C. achates* does not fly and is, therefore, a very slow disperser, considerable effort is needed to facilitate the population increase and distribution of the insect throughout the spotted knapweed-infested areas. Some mass rearing of the insect is being conducted in Montana (Story et al., 1996), but more effort of this type is needed. Efforts are also needed to identify those areas (i.e., habitat types or climatic zones) where *C. achates* may not be effective. Finally, studies on the integration of biocontrol with other management options are crucial. For example, grazing and reseeding of appropriate native plant species may prove to be important approaches to be used in conjunction with biological control.

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### Table 3

<table>
<thead>
<tr>
<th>Site</th>
<th>Plant species</th>
<th>2002</th>
<th>2004</th>
<th>Common name and characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Relative density ( % of total ± SE)</td>
<td>Absolute density (plants/m² ± SE)</td>
<td>Relative density ( % of total ± SE)</td>
</tr>
<tr>
<td><strong>TWR</strong></td>
<td><em>A. caninum</em></td>
<td>34 ± 5.3</td>
<td>65.5 ± 16.6</td>
<td>9 ± 2.5</td>
</tr>
<tr>
<td></td>
<td><em>B. tectorum</em></td>
<td>34 ± 8.7</td>
<td>52.6 ± 6.7</td>
<td>89 ± 2.6</td>
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<tr>
<td></td>
<td><em>C. stoebe</em></td>
<td>2 ± 1.2</td>
<td>—</td>
<td>1b</td>
</tr>
<tr>
<td></td>
<td><em>D. sophia</em></td>
<td>18 ± 3.3</td>
<td>37.1 ± 11.1</td>
<td>1b</td>
</tr>
<tr>
<td></td>
<td><em>Erodium cicutarium</em> (L.) L'Hér.</td>
<td>11 ± 2.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><em>P. pratensis</em></td>
<td>1b</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>LMR</strong></td>
<td><em>A. caninum</em></td>
<td>25 ± 6.0</td>
<td>67.2 ± 13.2</td>
<td>26 ± 10.3</td>
</tr>
<tr>
<td></td>
<td><em>Agropyron cristatum</em> (L.) Gaertn.</td>
<td>1b</td>
<td>—</td>
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</tr>
<tr>
<td></td>
<td><em>B. tectorum</em></td>
<td>10 ± 3.1</td>
<td>—</td>
<td>50 ± 8.8</td>
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<tr>
<td></td>
<td><em>C. stoebe</em></td>
<td>10 ± 3.0</td>
<td>—</td>
<td>9.5 ± 4.4</td>
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<tr>
<td></td>
<td><em>D. sophia</em></td>
<td>29 ± 5.0</td>
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<td>24 ± 4.0</td>
<td>73.1 ± 19.8</td>
<td>12 ± 5.3</td>
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<td>—</td>
<td>0.5b</td>
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</table>

* a The absolute density was only presented for species with >30 individuals in the total sample.  
  b Standard error not produced due to insufficient number of individuals.
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