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Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canada

Judith H. Myers [a,*], Caroline Jackson [a], Hillary Quinn [a], Shannon R. White [a], Jenny S. Cory [b]

[a] *Departments of Zoology and Agroecology, University of British Columbia, 6270 University Blvd., Vancouver, BC, Canada V6T 1Z4*
[b] *Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Simon Fraser University, Burnaby, BC, Canada V5A 1S6*

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ABSTRACT

The biological control program for diffuse knapweed, *Centaurea diffusa* Lamarck, a Eurasian plant that has invaded large areas of grasslands in western North America, has gone on for over 35 years. This program involved the release of 12 biological control agents of which four are numerous and widely distributed; two species of Tephritid flies, *Urophora affinis* and *Urophora quadrifasciata*, the root boring beetle, *Sphenoptera jugoslavica*, and the most recently established weevil *Larinus minutus*. Field observations show that diffuse knapweed densities declined at sites in British Columbia Canada where the weevil *L. minutus* became established. Decline in diffuse knapweed density did not occur where densities of *L. minutus* were low. Field cage experiments showed that feeding by *L. minutus* damaged rosette leaves and bolting stems, and reduced seed production, seedling density and the density of rosette and flowering diffuse knapweed plants.

1. Introduction

Introduced invasive weeds are changing plant communities around the world (Myers and Bazely, 2003). The only way to reduce their densities on a large spatial and temporal scale is through biological control; the introduction of natural enemies from the native habitat of the plant to the exotic habitat where it has become problematic. Biological control programs for weeds are large experiments in insect–plant dynamics. Why do some insect species reduce the densities of their hosts while others do not? Predicting what type of agents will be successful continues to be a challenge.

Most of the funding for biological control programs is targeted at finding, host specificity testing, and releasing agents while little support is available for post release evaluations (Ding et al., 2006). The evaluation of successful biological control programs is important however, as it may indicate the types of agents that are most likely to be successful. With calls for greater emphasis on the efficacy of biological control agents (McClay and Balciunas, 2005) it is important to evaluate the impacts of agents that have been released and their characteristics. If the potential for success of particular agents could be predicted in advance, the number of introductions could possibly be reduced, and thus the overall risk associated with adding more foreign species to new environments would also be reduced (Louda et al., 1997, 2003).

Here both long- and short-term field data are evaluated for the biological control of diffuse knapweed, *Centaurea diffusa* Lam., in British Columbia, Canada. Field cage experiments are also used to explore the impact of the most recently established agent, *Larinus minutus* Gyll., on diffuse knapweed density.

Diffuse knapweed is of Eurasian origin and was introduced to North America in the early 1900s, and has since spread to over a million hectares of rangeland in western Canada and the United States (Story et al., 2000; LeJeune and Seastedt, 2001). Diffuse knapweed is a short-lived perennial plant with seed germination occurring in the spring and autumn associated with rain. Rosette plants develop over the spring and if they reach a sufficient size, they bolt and flower in May and June, or remain as rosettes until the next year (Powell, 1988). Knapweed is a serious rangeland weed because it is poor forage for cows and displaces grasses (Harris and Cranston, 1979).

Since 1970, 12 species of insects have been introduced for biological control (Bourchier et al., 2002) and 10 have become established. In the early years of the knapweed biological control program considerable effort went into the evaluation of the impacts of biological control species in British Columbia, Canada (Roze, 1981; Morrison, 1987; Powell, 1988). These studies showed that agents that merely reduce seed production were not sufficient to reduce plant density.

Of the species introduced for the biological control of diffuse knapweed four are now widespread and abundant; two species of Tephritid flies, *Urophora affinis* Frfld. and *Urophora quadrifasciata*

* Corresponding author. Fax: +1 604 882 2416.
E-mail address: myers@zoology.ubc.ca (J.H. Myers).

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Meigen., introduced in the early 1970s, the root boring beetle, *Sphenoptera jugoslavica* Obenb., introduced in the late 1970s, and the most recently established agent, the weevil *L. minutus* (Groppe, 1990). *Larinus minutus* was initially introduced between 1996 and 1999 and then redistributed to 230 new sites in British Columbia in 2000 and 2001 (BC Ministry of Forests, PENWEED reports).

Larvae of both *Urophora* spp. and *L. minutus* develop in the flower heads. The adults of the two fly species emerge from seedheads and are active in May and June when females lay eggs in the flower buds and larvae form galls at the base of the flower head. A proportion of the larvae may complete development and emerge as adults later in the summer and the remainder emerge as adults the next spring.

In May, *L. minutus* adults emerge from the soil where they have over-wintered. Over the next month they mate and feed on the leaves, stems and buds of knapweed plants. Females of *L. minutus* oviposit in flower heads from late May to July and larvae develop in the seedheads, one per head. Adults emerge in late summer when they feed on the knapweed plants before moving into the soil to over-winter.

Adult *S. jugoslavica* emerge from roots of knapweed plants during July to August, and oviposit in the root crown of rosettes. The larvae develop in the roots through the early autumn, and then complete their development during the following spring before emerging as adults in summer.

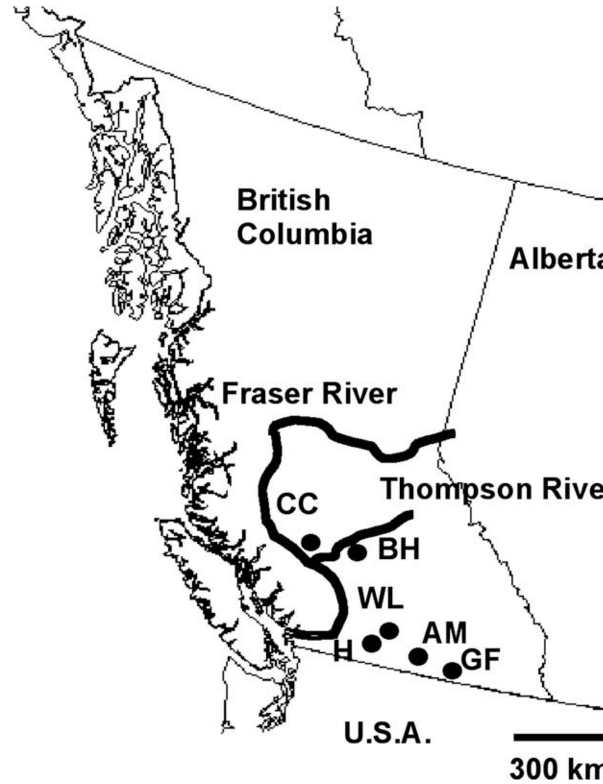

Fig. 1. Schematic map of British Columbia showing the locations of the study sites. CC, Cache Creek; BH, Buse Hill; WL, White Lake; H, Hedley; AM, Anarchist Mountain; and GF, Grand Forks.

2. Methods

2.1. Field sites

All of the field sites, both long- and short-term, (Fig. 1) were in the Ponderosa Pine – Bunchgrass Biogeoclimatic zones except Buse Hill, which is in the Interior Douglas Fir zone. The grasslands of BC are relatively diverse with over 100 plant species having been identified in pastures in the vicinity of White Lake (Stephens, Krannitz and Myers, unpublished). Common to the grassland plant community at these sites are the grasses, needle-and-thread grass (*Heterostipa comata* (L.) Trin. & Rupr.), Sandberg bluegrass (*Poa secunda* J. Presel), red three-awn (*Aristida longiseta* Steud.), and bluebunch wheatgrass (*Pseudoregneria spicatus* (Pursh)). Crested wheat grass, (*Agropyron cristatum* (L.) Gaertn.), was planted at the Cache Creek site approximately 40 years ago and remains common. Cheatgrass (*Bromus tectorum* L.) appeared to be increasing following the decline of knapweed at the sites (personal observation). Bare ground was common at all sites.

2.2. Monitoring sites and procedures

Diffuse knapweed density and biological control agents were monitored at four long-term sites in British Columbia from the mid to late 1970s to 1994, and from 2003 to 2008. At one of these sites, White Lake, plant density was also monitored in 1999 and 2001 (Table 1). From 2004 to 2008 knapweed was monitored at an additional site; Anarchist Mountain with a high-density of diffuse knapweed but low *L. minutus* density, and from 2005 to 2008, at Hedley, where knapweed density was initially observed to be high as compared to other sites where it had already declined.

To monitor knapweed at the four long-term study sites flowering stems, rosettes and seedlings were counted in 50×50 cm quadrats (2500 cm^2) along arbitrarily chosen transects that covered the same general areas in each year. Quadrat frames were dropped every 10 paces as an observer walked with their eyes closed to avoid bias in the quadrat placement. In most years arbitrary samples of both rosette and bolted plants were uprooted and the roots were sliced open with a knife to determine if they were attacked by *S. jugoslavica, Agapeta zoegana* L. a root boring Lepidoptera or *Cyphocleonus achates* Fahraeus, a root boring weevil.

Typically plants were counted in 30 quadrats at each site in late July or August. Following the reduction of knapweed at some monitoring sites, it was necessary to evaluate biological control agents on the few remaining plants in the general vicinity of the traditional monitoring sites.

2.3. Cage experiments

In 2003 a wild fire burned a large area of grassland and woodland along MacIntyre Creek Road on the east side of Vaseux Lake, BC. In 2004 diffuse knapweed density was extremely low (no flowering stems in ninety-six 0.25 m^2 quadrats). It was anticipated that knapweed would return to this area and thus provide an opportunity for evaluation of the impact of the biocontrol agents. By 2005 diffuse knapweed had indeed increased from the surviving seed bank and the site became suitable for an experiment to evaluate the impacts of *L. minutus*.

Three replicates of each of three treatments; caged *L. minutus*, caged control and uncaged control, were established in three sites that were approximately 500 m apart, called Lower, Middle and Upper Meadow. Areas with similar densities of flowering stems were found for placement of experimental cages. The number of flowering stems was kept constant among treatments at each site through stem removal and were; Lower Meadow = nine stems/cage, Middle Meadow = 17 stems/cage and Upper Meadow = 13 stems/cage. Each replicate of three treatments was contained within a radius of approximately 15 m and depended on patches having sufficient knapweed. Once the plots were established treatments were assigned at random.

Table 1
Geographic coordinates of study sites, elevations, slope grazing history and precipitation averages during the knapweed growing season for 1971–2000 based on the nearest weather station and obtained from Environment Canada (http://climate.weatheroffice.ec.gc.ca/climate_normals/index_e.html accessed 14 November 2005). Na, no weather stations are near Anarchist Mt., it is likely to receive more rain than White Lake because of its higher elevation.

Site	Coordinates	Elev. (m)	Av. precipitation May to July (mm)	Slope	Grazing
White Lake	49° 19 N 119° 37 W	550	104	Level	No
Grand Forks	49° 00 N 118° 17 W	519	155	South facing	No
Cache Creek	50° 50 N 121° 23 W	512	88	East facing	No
Buse Hill	50° 36 N 120° 02 W	623	179	South facing	Summer
Anarchist	49° 00 N 119° 17 W	1085	NA	South facing	Autumn
Vaseux Lk.	49° 18 N 119° 31 W	377	104	West facing	No
Headley	49° 20 N 120° 04 W	534	103	Level	No

The cages were 0.25 m^2 in area, 1 m tall and constructed from 1/2 inch PVC pipe covered by nylon window screening. The cages were secured by attaching them with wire to 30-cm long spikes in each of the four corners. The netting fringe was pegged down with twelve 10-cm nails around the perimeter. *Larinus minutus* were collected from high-density sites in the vicinity and six beetles per flowering stem were released into treatment cages on 12–13 June, 2005 when the flowering stems had recently bolted. This density compared with those typically observed in the open, i.e. from 2 to 12 beetles per plant might be observed on plants at any one time.

On July 18, 2005 the cages were temporarily removed and flowers were hand pollinated using flowers from nearby plants outside the cages. At this time leaf damage from beetle feeding was assessed as the percentage damage to leaves and stems of individual plants.

In late August plants were sampled by removing three branches from the lower, middle and top sections of each flowering plant. These were placed in bags and taken for inspection in the laboratory where all developed heads were removed and a sample of 50 were opened and scored for the presence of *L. minutus* larvae and whether any seeds had developed in the head.

Cages were removed in the September of 2005 to avoid damage over-winter, and replaced on 12 May, 2006. The numbers of seedlings, rosettes and bolting plants were counted before the cages were replaced in the same location. Rosettes and bolting plants were combined for analysis because not all of the rosettes that could have potentially bolted that spring had done so. The number of seedlings, rosettes and bolting plants were also counted on 17 August, 2006 and the seed heads were sampled as in 2005. The presence or absence of *Urophora* spp. flies was also noted. Some quadrats had no flowering plants in 2006.

Similar cage experiments were established at Anarchist Mountain, a site with high knapweed and *S. jugoslavica* densities and low *L. minutus* density in 2005. This site is at a higher elevation than the Vaseux Lake site (Table 1) and is approximately one month later in terms of phenology. Cages were the same as those described above and six groups of three treatments were established; *L. minutus* addition, six per flowering plant added in July, caged controls and uncaged controls. The impact of the *L. minutus* releases on seed production, *L. minutus* density and leaf feeding damage were assessed in August 2005 but plant densities were not. In May 2006 the marking pegs were found to have been removed by an outside agency and thus cage locations could not be identified to evaluate plant densities.

2.4. Statistical analysis

The impacts of *L. minutus* on knapweed in the cage experiments were compared using a one-way analysis of variance in JMP 5.1.2. Proportions were arcsin transformed and the numbers of plants and damage levels were square root transformed to increase the fit to a normal distribution before analysis. Annual densities of stem plants before and after the establishment of *L. minutus* were compared with the nonparametric Kruskal–Wallis test using JMP 6.

3. Results

3.1. Distribution of biological control agents and plant densities

The *Urophora* gall flies occurred at all sites following their widespread manual distribution and establishment in the 1970s (Table 2). Knapweed densities in the presence of *Urophora* spp. between the 1970s and mid-1990s showed no apparent decline in plant density (Fig. 2) even though the flies reduced knapweed seed production substantially (Powell and Myers, 1988; Powell, 1990).

Sphenoptera jugoslavica also occurred at all the monitoring sites (Table 2). This species was established by 1978 at White Lake and, between then and 2001, an average of 45% (SE 5, $n = 12$ years) of plants had damaged roots or beetle larvae in the roots. We first observed *S. jugoslavica* beetles at Grand Forks in 1989 and recently, on average 40% (SE 5, $n = 4$ years) of roots have been damaged or contain beetles. This species was also first observed at Cache Creek and Buse Hill in 1989. Following the establishment of, *S. jugoslavica*, diffuse knapweed populations at White Lake, Grand Forks and Buse Hill showed no declining trends during the 1980s through

Table 2
Occurrence of biological control agents at diffuse knapweed field sites.

Site	*Larinus*[a]	*Agapeta*[b]	*Cyphocleonus*[b]	*Sphenoptera*[b]	*Urophora* spp.[c]
White Lake	0.34 (0.10) 3	0	0.1 (0.1–0.2)	0.2 (0.1–0.3)	0.9 ± 0.2
Cache Creek	0.47 (0.06) 4	0.04 (0–0.1)	0	0.6 (0.4–0.7)	0.5 ± 0.1
Grand Forks	0.29 (0.04) 4	0.10 (0–0.4)	0.04 (0–0.2)	0.3 (0.1–0.6)	1.1 ± 0.2
Hedley	0.62 (0.10) 2	0	0.4 (0.2–0.6)	0.2 (0.0–0.4)	0.3 ± 0.1
Anarchist Mtn.	0.04 (0.02) 2	0	0	0.7 (0.7–0.8)	0.7 ± 0.1
Buse Hill	0.08 (0.08) 4	0	0	0.3 (0.3–0.4)	2.3 ± 0.3

[a] Mean proportion of seed heads attacked by *L. minutus* (±SE) N (years sampled).
[b] Either agents or their damage in roots (binomial 95% confidence intervals) in 2007.
[c] Mean ± SE number of *Urophora* spp. galls in seed heads in 2007. Most of the galls were *U. affinis*.

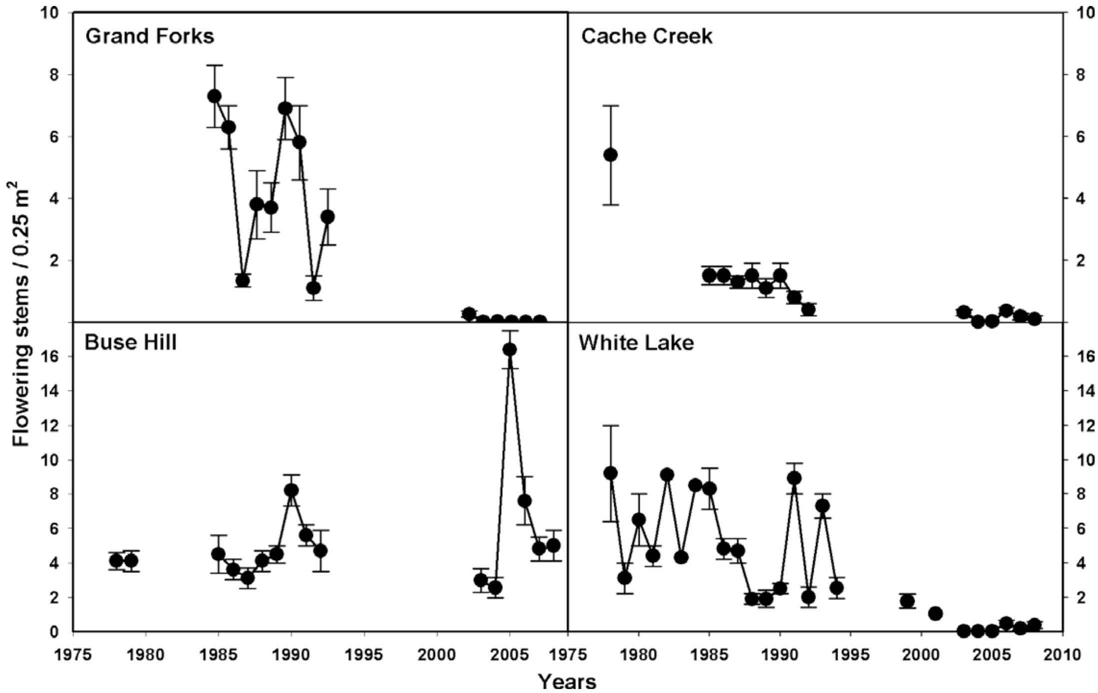

Fig. 2. The mean (±SE) density per 0.25 m^2 of diffuse knapweed flowering stems at four long-term field sites in British Columbia. *Larinus minutus* were introduced in the vicinity of these sites in the late 1990s but have not become common at Buse Hill (see Table 2).

mid-1990s in agreement with the study by Powell (1990). A possible exception to this pattern was seen at Cache Creek where densities of knapweed declined from 1989 to 1994 (Fig. 2).

Agapeta zoegana and *C. achates* occurred at low densities at Grand Forks and White Lake. Highest densities of *C. achates* were found in 2007 in the few plants remaining at Hedley following the knapweed decline. *Cyphocleones achates* was not observed at Cache Creek, Buse Hill or Anarchist Mtn. (Table 2).

In 2003 and 2004 study areas fell into two categories; those with obvious adult *L. minutus* beetles or damage to plants (White Lake, Cache Creek and Grand Forks) and those with no or very few adult beetles and no observed damage (Buse Hill and Anarchist Mountain) (Table 2). *Larinus minutus* was most numerous at Grand Forks and White Lake with an average of over two adult beetles observed per plant in July 2004 at those sites.

The decline in diffuse knapweed densities for the three long-term sites with *L. minutus* was striking (Fig. 2). Densities of flowering stems were significantly lower (based on Kruskal Wallis non-parametric comparisons) from 2003 to 2008 than in the years prior to the establishment of *L. minutus* (1978 to 1994 for Cache Creek ($\chi^2 = 11$, $P < 0.001$), 1985 to 1994 for Grand Forks ($\chi^2 = 11$, $P < 0.001$), and 1978–1999 for White Lake ($\chi^2 = 14$, $P < 0.001$).

At Buse Hill, where *L. minutus* was first observed in 2005 and remained low in 2007 (Table 2), the average density of plants from 2003 to 2006 was not different from the average of previous years, 1978 to 1994 ($\chi^2 = 0.6$, $P = 0.44$). Diffuse knapweed densities declined to zero at the Hedley site with high levels of attack by *L. minutus* between 2005 and 2006 (Fig. 3). At Anarchist Mountain densities of *L. minutus* remained low (Table 2) and diffuse knapweed density remained high (Fig. 3). Half of the plants at this site had *S. jugoslavica* larvae or evidence of previous attack in 2004, and in 2007 approximately three quarters of the plants had damaged roots (Table 2).

3.2. Experimental results

In 2005, at both experimental locations (Anarchist and Vaseux Lake), the cages to which *L. minutus* were added produced a significantly higher proportion of *L. minutus* per seed head during the summer than the caged controls (Fig. 4). A low level of *L. minutus* attack also occurred in the caged control plots, indicating that a small number of beetles entered these cages. The proportion of heads with viable seeds was significantly reduced in plants caged with *L. minutus* (Fig. 4). Finally, at both study sites, the average percentage of feeding damage to the leaves was significantly higher for plants in cages with *L. minutus* than those lacking beetles (Fig. 4).

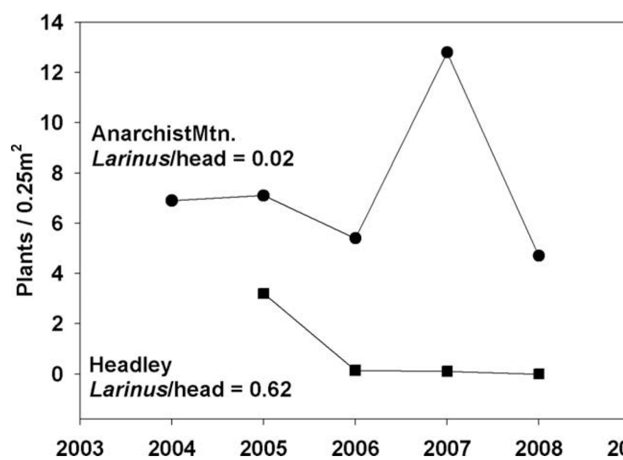

Fig. 3. The mean density per 0.25 m^2 of diffuse knapweed flowering stems at two short-term field sites in British Columbia. *Larinus minutus* were introduced in the vicinity of both of these sites and became common at Hedley in 2006 but have never become common at Anarchist Mountain (see Table 2).

In the following spring, May 2006, the average densities of seedlings ($F_{2,25} = 4.8$, $P < 0.02$) and rosettes and bolting stems ($F_{2,25} = 3.5$, $P < 0.05$) were lowest in cages with *L. minutus* at the Vaseux Lake site (Fig. 5). By the end of the summer in 2006 all of the cages at Vaseux had been invaded by *L. minutus* and treatments were no

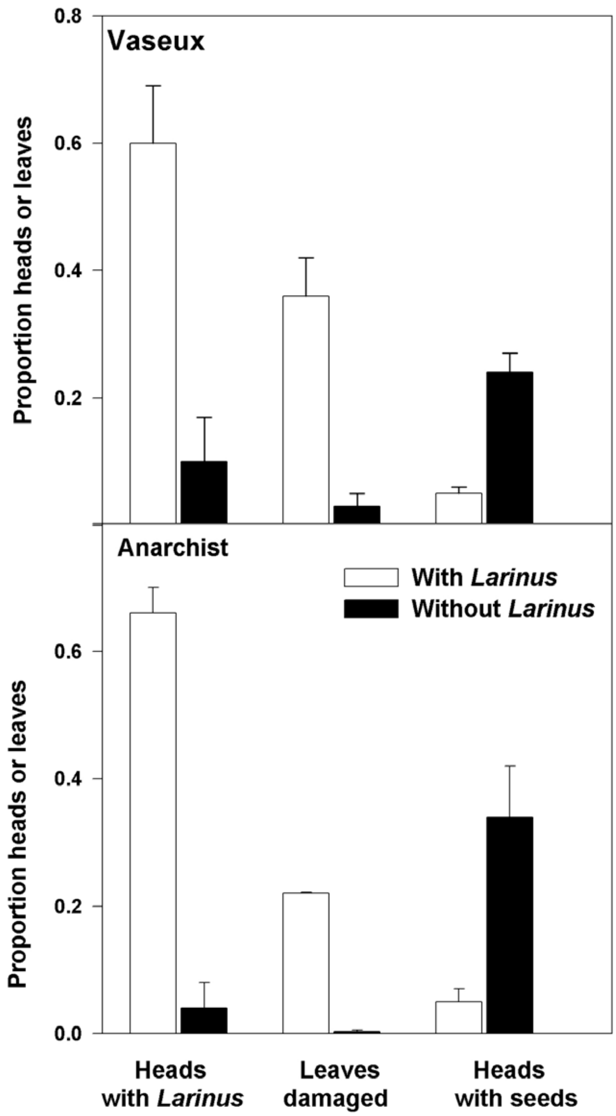

Fig. 4. Means (±SE) of the proportion of diffuse knapweed heads with *L. minutus*, proportion leaves damaged, and proportion of heads with seeds in the cage experiments at the Vaseux Lake and Anarchist Mountain in August 2005. Means for plants in experimental cages with or without *L. minutus* are significant in all cases. Vaseux ($F = 18.2, 29, 31$; df = 1,16; $P < 0.001$ for all cases). Anarchist ($F = 12.2, 17.6, 11.3$; df = 1, 10; $P < 0.01$ for all cases).

longer distinguishable based on the proportion of seedheads attacked by *L. minutus* ($F = 1.41$, df = 2.25, $P = 0.24$). The seedheads from uncaged control plants had the highest proportion (mean ± SE: 0.6 ± 0.1) of heads with *L. minutus* showing that the biological control agents were moving back to this previously burned site. The *L. minutus* treatment and caged controls had the same proportion of heads attacked by *L. minutus* (mean ± SE: 0.42 ± 0.09 and 0.41 ± 0.05, respectively). No *Urophora* spp. was found in the seedheads from caged plants in either 2005 or 2006.

The similar levels of attack by *L. minutus* among treatments were reflected in the plant densities in the autumn of 2006 at the Vaseux sites. The average number of seedlings was lowest in the *L. minutus* treatment, but this was only significantly different from the number of seedlings in the caged control ($F_{2,25} = 8.5$, $P = .002$) (Fig. 6). The average number of rosettes and flowering plants was lowest for the uncaged controls which by then had high levels of attack by *L. minutus* and were similar to the *L. minutus* treatment (Fig. 6).

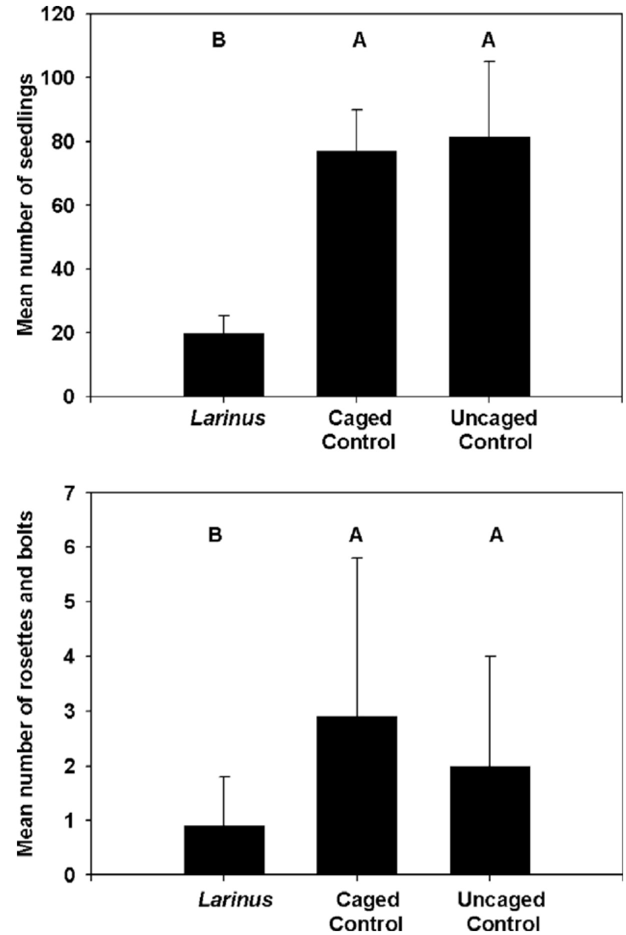

Fig. 5. Means (±SE) of the number of rosettes and bolting stems and of the number of seedlings per 0.25 m^2 in the cage experiments at the Vaseaux Lake site in May 2006. Experimental treatments are uncaged control, caged control, and *Larinus* addition: sample size is 9 in all treatments except C aged control, which has 8 due to disturbance of the cages. Letters above the bars indicate treatments that are significantly different at $P < 0.05$ (least squares means difference, student's *t*).

4. Discussion

The successful biological control of diffuse knapweed was slow, 30 years and 12 agents introduced. The first three biological control species to be introduced, the two *Urophora* spp. and *S. jugoslavica*, reduced seed production dramatically, but did not kill plants or reduce plant density. Simulation models have shown that seed reduction is not sufficient if seedling survival is compensatory (Myers et al., 1990; Powell, 1990; Myers and Risley, 2000; Myers and Bazely, 2003). These predict that for success, an agent must have impacts on other life stages of plants and kill them (Myers and Risley, 2000).

Adult *L. minutus* feed on leaves of rosettes and seedlings and the parenchyma of bolting stems. The impact of this on plants is particularly damaging in dry summers, such as 2003, and in exposed locations with sandy soil (Jackson and Myers, personal observation). Their feeding damage can kill plants. In addition, larvae develop in the buds, eliminating seed production in attacked flower heads. *Larinus minutus* meets the predicted characteristics of a successful biological control agent.

Although the initial declines in knapweed density occurred in dry summers, densities have increased only slightly in years of more normal rainfall (Fig. 7). That diffuse knapweed density normally fluctuates with rainfall has been shown previously (Myers and Bazely, 2003).

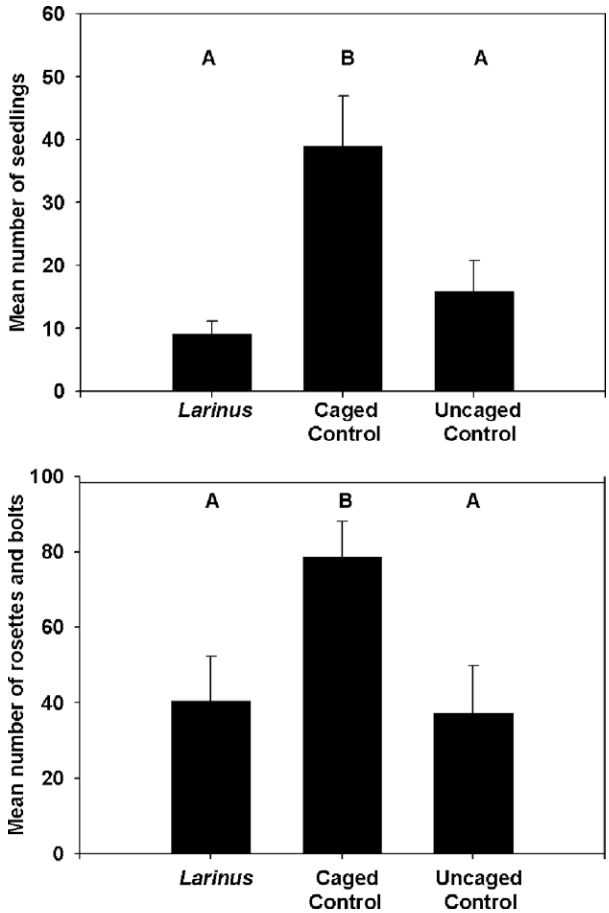

Fig. 6. Means (±SE) of the number of rosettes and bolting stems and of the number of seedlings per 0.25 m^2 in the cage experiments at the Vaseux Lake site in August 2006. Experimental treatments are uncaged control, caged control, and *Larinus*: sample size is 9 in all treatments except C, which has 8 due to disturbance of the cages. Letters above the bars indicate treatments that are significantly different at $P < 0.05$ (least squares means difference, student's t).

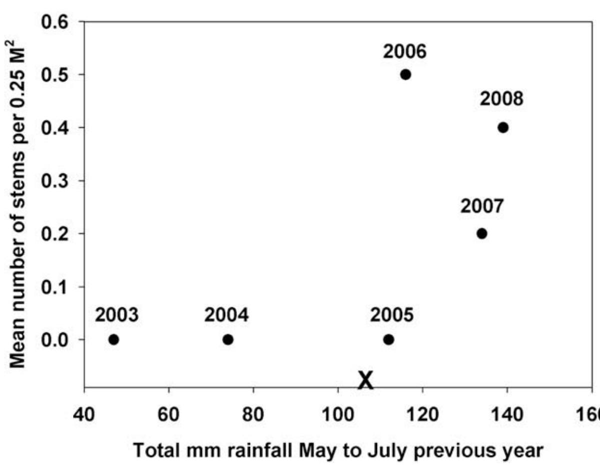

Fig. 7. Total mm rainfall in May through July (knapweed main growing season) and density at the White Lake site since knapweed densities declined. Years are identified on the figure and *X* indicates the average spring rainfall between 1978 and 2008 based on records from Environment Canada, Penticton BC weather station.

The failure of seed reduction to translate into density reduction has also been shown by Story et al. (2008) in a recent study in Montana of the biological control program for spotted knapweed, *Centaurea stoebe* L. subsp. *micranthos* (Gugler) Hayek. This study focused on the reduced seed production caused by *Urophora* spp. and *Larinus* spp. The levels of reduced seed production of 90–95% are similar to those that occurred 20 years ago for diffuse knapweed in British Columbia (Powell, 1990) without a decline in plant density. Story et al. (2008) concluded that because the relationship between stem density and seed bank density is non-linear, the seed bank will need to fall below a critical threshold before plant density will decline. This is equivalent to the results of the models for diffuse knapweed that led to the prediction that to be effective, an agent must kill plants at a stage after which compensatory survival will not occur (Myers and Risley, 2000; Powell, 1990).

Story et al. (2008) do not mention *L. minutus* feeding on other life stages of spotted knapweed plants and causing other types of damage. Reductions of spotted knapweed however, have occurred in some areas of Montana associated with attack by *C. achetes*, a root-feeding beetle that can kill plants (Corn et al., 2006; Story et al., 2006). For spotted knapweed therefore, a reduction in the seed production is not sufficient to reduce plant density but increased mortality of plants caused by the root-feeding weevil, *C. achetes* appears to be so.

The decline in diffuse knapweed density in the dry interior of British Columbia in the last eight years has not only occurred at monitored sites. Knapweed densities have also declined at many locations and along roadsides that formerly were highly infested (Myers, personal observation). This biological control success following the establishment of *L. minutus* extends beyond British Columbia. At two sites in Montana flowering stem densities of diffuse knapweed declined 76% and 90% between 1998 and 1999 (Smith, 2004). At three sites in Colorado, flowering plant densities declined by 99% (1997–2003) and 95% and 83% (2001–2003) (Seastedt et al., 2003).

Up to six different species of biological control agents can be found at the field sites in BC (Table 2). The potential interactions of *L. minutus* with other agents have been tested with differing results in other studies. Smith and Mayer (2005) used field cages to study the interactions of *L. minutus* and the gall fly *U. affinis* on both diffuse and spotted knapweed. They found that *L. minutus* reduced the reproductive success of *U. affinis* by 71% on spotted knapweed, *C. stoebe*, and 77% on diffuse knapweed. Crowe and Bouchier (2006) found the opposite with the use of enclosure and exclosure cages and spotted knapweed; attack rates by *L. minutus* were reduced by the presence of *U. affinis*. These differing results may have been caused by variation in the phenology of the plants in the two studies. In field observations in Colorado, Seastedt et al. (2007) found that *Urophora* spp. and *L. minutus* in flower heads of diffuse knapweed fluctuated independently, and their data indicate that *S. jugoslavica* persists at field sites in the presence of *L. minutus*. Overall it appears that multiple agents can persist in this system.

A majority of weed biological control successes have been attributed to single species of agents although different species may be required for success under different environmental conditions (Denoth et al., 2002; Myers, 2008). Further work is necessary to determine the constraints on the effectiveness of *L. minutus*. It is possible that *L. minutus* will not be as successful and will not reduce the density of diffuse knapweed in upper elevation and moister locations and another agent will be necessary for this type of habitat. The phenology of the beetles and the development of diffuse knapweed plants may not be synchronized at higher elevations preventing the beetle populations from increasing as well as at lower elevation, warmer and drier sites. In addition the attack of *L. minutus* on rosettes and bolting plants may be less damaging at moister, cooler, higher elevation sites. The sister species *L. obtusus* Gyllenhal has been introduced in British Columbia on spotted knapweed and it may do better on diffuse knapweed at these higher elevation sites.

Identifying potentially successful agents remains a challenge. It has been suggested that successful agents will be those that are rare in their native habitat e.g., those natural enemies to which the host plant will have not adapted (Myers, 2001). Little is recorded about the status of *L. minutus* in Europe. That it was the last of 12 species of biological control agent to be introduced to Canada may indicate that it is not common in its native habitat. Groppe (1990) recorded that *L. minutus* occurred at only a few of the European sites that were surveyed, but when present, was the dominant flower head insect. She did not mention in the initial European studies whether adult beetles killed plants, but did note that they feed on green plant tissue. Cage experiments similar to those reported here might have identified the potential of *L. minutus* to be a successful agent if they had been carried out in the native habitat in the initial studies of this species.

In conclusion, the following observations support the interpretation that diffuse knapweed has responded to successful biological control in British Columbia: (1) the decline in diffuse knapweed densities following the establishment and spread of high densities of *L. minutus*, (2) the decline in knapweed density at sites with high numbers of *L. minutus* and the continued high knapweed density at sites with low numbers of this beetle species, (3) the continued low densities of knapweed at sites with *L. mintus* for 6 years following the initial population decline and (4) the reduced density of knapweed in experimental cages with *L. minutus* compared to those without this biological control agent.

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