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

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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.

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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).

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





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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.

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 cm²) 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



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.

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 m² 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.

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