

**POTENTIAL ENVIRONMENTAL INFLUENCES ON BIOLOGICAL  
CONTROL: CAN DROUGHT IMPROVE SUCCESS AND DO CONGENERS  
PREFERENTIALLY EXPLOIT DIFFERENT HABITATS?**

**by**

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## Abstract

Climate change is a major threat to global biodiversity, and will alter species ranges, plant and animal phenology, and species interactions within ecosystems. Climate mediates plant-insect interactions, and consequently has the potential for positive or negative effects on biological control systems. Observational evidence suggests that a recent dramatic reduction in the density of diffuse knapweed, *Centaurea diffusa* Lamarck, in sites in British Columbia, Canada is attributed to the biological agent *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae). This decline took place over several years of late spring and summer drought which suggested an association between dry conditions and successful biological control. To explore this, I conducted field experiments using rain shelters and watering treatments to assess the effectiveness of plant attack by *L. minutus* under moist and dry conditions. I found that *L. minutus* reduced seed production regardless of moisture conditions, with a trend towards greater seed reduction under dry conditions.

Two or more species of insects in the same genera have been introduced in some weed biocontrol programs. If the species are ecological equivalents and compete the introduction of both species may be detrimental and reduce their impacts. If however the species vary in their distributions, the introduction of congeners may be advantageous. I review the following cases of species pair releases: the beetles *Chrysolina quadrigemina* (Suffrian) and *Chrysolina hyperici* (Forster) for St. John's wort (*Hypericum perforatum* L.); the gallflies *Urophora affinis* Frfld. and *Urophora quadrifasciata* (Meig.) for *Centaurea* species; the weevils *Neochetina bruchi* Hustache and *Neochetina eichhorniae* Warner for water hyacinth (*Eichhornia crassipes* (Mart.) Solms.) and the beetles *Galerucella pusilla* Duftschmidt and *Galerucella californiensis* L. for purple loosestrife (*Lythrum salicaria* L.). I found that congeneric agents can offer complementary control of target weeds in slightly different habitats. Given the increasing focus on possible non-target effects of biological control introductions, I recommend that greater care be taken to avoid mixed species introductions and that judicious use be made of controlled field experimentation to determine species impacts. Molecular studies of species before introduction could help prevent the accidental introduction of multiple species.

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### **Co-authorship statement**

Chapter 3 has been accepted for inclusion in the Proceedings of the XII International Symposium on the Biological Control of Weeds, Montpellier, France (CABI, Wallingford, UK, 2008). I conducted the majority of the research and wrote the paper, while J.H. Myers assisted with study design and improved the manuscript.

## Chapter 1: General introduction

Climate change is a major threat to global biodiversity (Hughes 2000; McLaughlin et al. 2002; Parmesan & Yohe 2003), and will alter species ranges (Thomas et al. 2004), plant and animal phenology (van Asch et al. 2007), and species interactions within complex ecosystems (Davis et al. 1998). The spread of non-native species presents an additional threat (Czech & Krausman 1997; Wilcove et al. 1998). The formidable challenge for land managers today is conserving native biodiversity while responding to increased disturbance regimes and global change (Myers & Bazely 2003).

Management goals for invasive species differ from those for native species: the aim is to reduce the population of the invasive rather than to conserve it. How a changing climate will affect current strategies and tools used for invasive species management is a problem that has not received much attention, and researchers have recently highlighted the need to integrate biological invasion and climate change biology (Hellmann et al. 2008). Biological control, the use of natural enemies to reduce the impact of invasive plants, provides a long-term solution to the management of alien plant species. Efficacy depends on factors such as climate, target weed phenology, nutrient conditions, dispersal ability, fecundity, and type and level of damage to key life stages of the plant. All of these parameters could be affected either directly or indirectly by changes in temperature, precipitation, or carbon dioxide levels under global change (review in Ward & Masters 2007).

### **How does precipitation affect biocontrol of *Centaurea diffusa* by *Larinus minutus*?**

Diffuse knapweed (*Centaurea diffusa* Lamarck) is a plant of Eurasian origin that has invaded grasslands of western North America. The plant produces a large number of seeds, and has a flexible life cycle, with the ability to remain as a rosette for a few years before flowering (Powell 1990; Myers & Bazely 2003). Twelve species of insect biological control agents have been introduced that attack diffuse knapweed (Bourchier, Mortensen & Crowe 2002; Myers 2007). Observational evidence suggests that a recent

reduction in diffuse knapweed density in the Okanagan Valley of British Columbia was associated with the establishment of the most recently released biological agent, the seedhead weevil *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) (Myers 2007). Because the knapweed decline was observed the same year in which a severe late spring and summer drought took place (J.H. Myers, unpublished data), this suggests that environmental factors could influence the impact of *L. minutus* weevils on the plants.

The plant stress hypothesis (White 1969, 1984) predicts that increased insect herbivore performance and population outbreaks during drought conditions can be attributed to changes in foliar nitrogen. Under water deficit conditions, protein synthesis is impaired and tissue nitrogen content increases (Hsiao 1973). The increase in nitrogen availability for nitrogen-limited herbivores therefore results in increased insect performance. In contrast, Price (1991) proposes the plant vigour hypothesis, that herbivorous insects feed preferentially on more vigorous plants or plant nodules, while Huberty and Denno (2004) propose the pulsed stress hypothesis: drought stress alternates with periods of increased water availability and turgor recovery, thus providing insects with the access to the increased free nitrogen in plant tissues.

In Chapter 2, I present the results of field experiments using constructed rain shelters and weekly watering treatments to assess the effectiveness of *L. minutus* under moist and dry conditions. I considered the following questions: (1) does watering directly affect knapweed plant vigour; (2) does watering directly affect *L. minutus* survival and activity; and (3) does watering affect the consequences of *L. minutus* herbivory on knapweed?

I predict that the effect of *L. minutus* on diffuse knapweed would be greater in unwatered, drier plots, with shorter plants, reduced seed production, and lower bolting rates.

## **Are species pairs for the biological control of weeds advantageous or unnecessary?**

There is considerable debate in the literature regarding the degree of risk associated with biological control of invasive weeds (Frank 1998; Simberloff & Stiling 1996, 1998; Thomas & Willis 1998; Strong & Pemberton 2000). Choosing the most effective agents will reduce the potential for risk through nontarget effects (Louda et al. 2003).

Denoth et al. (2002) found that a majority of successes are attributed to a single agent. However, in some cases two species in the same genus have been introduced even though they are very similar in their interactions with host plants: this can be by accident or sometimes on purpose with species from different habitats. The competitive exclusion principle predicts that two similar sympatric species can coexist as they have some differences in niche requirements (Hardin 1960). If congeneric species act complementarily to reduce plant densities then their introductions would be warranted, and the greatest advantage would occur if different climatic tolerances of the species result in effective control of the host plant across different biomes.

In Chapter 3, I review congeneric releases worldwide, and examine four case studies to address the following questions: (1) is there evidence that both species are necessary for successful biological control; and (2) do congeneric species exploit different climatic regions and thus broaden the geographic effectiveness of biological control?

Finally, in the concluding chapter, I summarize the results of my research, offer suggestions for areas that warrant further investigation, and provide an overview of management implications.

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## **Chapter 2: Effect of watering on the impact of a seed weevil, *Larinus minutus*, in the biological control of an invasive knapweed, *Centaurea diffusa*<sup>1</sup>**

### **Introduction**

The spread of invasive alien species has contributed to biodiversity loss in terrestrial ecosystems (Czech & Krausman 1997; Wilcove et al. 1998). In addition, anthropogenic climate change can influence both the ranges of species and their interactions (Davis et al. 1998; Hughes 2000; McLaughlin et al. 2002; Parmesan & Yohe 2003). Biological control, the use of natural enemies to reduce the impact of invasive plants, is the only long term solution for the management of alien plant species. The interactions between plants and herbivores that are the basis for biological control could potentially be influenced by climate change in either positive or negative ways.

Diffuse knapweed (*Centaurea diffusa*) is a plant of Eurasian origin that has invaded the dry rangelands of western North America including the Okanagan Valley of British Columbia, one of the most unique and endangered ecosystems in Canada. Here diffuse knapweed reduced the productivity of rangeland by up to 90% (Watson & Renney 1974) and cost ranchers up to \$70/ha in lost forage (Harris & Cranston 1979). Observational evidence suggests that a recent dramatic reduction in the density of diffuse knapweed in sites in the Okanagan Valley was associated with the establishment of the biological agent *Larinus minutus*, a seedhead weevil (Myers 2007). This decline took place the same year in which the area experienced a severe late spring and summer drought (J. H. Myers, unpublished data) and this suggests that environmental factors could influence the impact of the weevils on the plants.

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<sup>1</sup>A version of this chapter will be submitted for publication: Jackson, C.A.R. Effect of watering on the impact of a seed weevil, *Larinus minutus*, in the biological control of an invasive knapweed, *Centaurea diffusa*



In this study, I conducted field experiments using constructed rain shelters and weekly watering treatments to assess the effectiveness of *L. minutus* under moist and dry conditions. The goal was to investigate how key demographic parameters of diffuse knapweed plants would respond to abiotic stress associated with drought conditions. To do this, I considered the following questions:

- 1) Does watering directly affect knapweed plant vigour as measured by plant height, seed production, flowering phenology, and bolting rate (proportion of rosette plants becoming bolting plants the following spring)?
- 2) Does watering directly affect *L. minutus* survival and activity as measured by weekly abundance, feeding damage to knapweed stems, and the numbers of *L. minutus* produced?
- 3) Does watering affect the consequences of *L. minutus* herbivory on knapweed, as measured by plant height, seed production, flower production, and bolting rate?

I predicted that the effect of *L. minutus* on diffuse knapweed would be greater in unwatered plots, and that these plots would have shorter plants with reduced seed production and lower bolting rates.

### **Biology of diffuse knapweed and *Larinus minutus***

Diffuse knapweed produces a large number of seeds and has a considerable seedbank. Seedling and rosette numbers are far more than what is necessary for replacement. Seedlings often emerge following rainstorms, and can emerge in the spring or in the autumn (Thompson & Stout 1991). Initially, plant resources are directed towards the growth of a large taproot, and very little above-ground biomass is produced other than a small, flat rosette. The plant has a flexible life cycle, with the ability to remain as a rosette for a few years before flowering (Myers & Bazely 2003; Powell 1990). Diffuse knapweed thus has two distinct morphological stages: the vegetative rosette stage, in which carbohydrates are produced and sequestered in a large taproot, and the flowering stage, where stored resources are converted into seeds.

Over the past three decades, 12 species of insect biological control agents have been introduced that attack diffuse knapweed (Bourchier, Mortensen & Crowe 2002; Myers 2007). Four root-feeders, the weevil *Cyphocleonus achates*, the moth *Agapeta zoegana*, the beetle *Sphenoptera jugoslavica*, and the moth *Pterolonche inspersa* have been introduced along with three seed feeders, two species of gall forming flies, *Urophora affinis* and *U. quadrifasciata*, and a flowerhead weevil *Larinus minutus*. Two fungi occur on knapweed, the indigenous *Sclerotinia sclerotiorum* and the introduced rust fungus *Puccinea jaceae*. Noticeable control is now emerging after the introduction of the most recent agent, *L. minutus*.

Adult *L. minutus* are capable of killing diffuse knapweed plants by feeding on the leaves, flowers, and stems. The larvae develop in the seed heads and reduce seed production. *L. minutus* beetles are univoltine. Adults overwinter in the plant litter within the knapweed patch. In May or June the weevils emerge and feed on the leaves, outer stem tissue, and flowers prior to laying eggs in newly-formed flowers (Groppe 1990; Kashefi & Sobhian 1998). In infested seedheads, seed destruction is 100%. New adults emerge and feed on the remaining vegetative portions of the host plants until the end of August, and then move to overwintering sites in the soil at the base of the plants in September (Kashefi & Sobhian 1998).

## Methods

### Study sites

Experiments were replicated at three sites in the South Okanagan Valley of British Columbia, Canada. Sites 1 and 2, Lower MacIntyre (49° 17'57'' N, 119° 31'40'' W, elevation 366 m) and Upper MacIntyre (49° 17'42'' N, 119° 31'26'' W, elevation 388 m), are 22 km south of Penticton, located along MacIntyre Road, 0.5 and 1.5 km respectively from Highway 97. These sites are in the lower elevation Bunchgrass biogeoclimatic zone as defined by the B.C. Ministry of Forests (Meidinger and Pojar 1991). Dominant grasses at the Lower MacIntyre site include needle-and-thread grass (*Heterostipa comata*), Sandberg bluegrass (*Poa secunda*), and cheatgrass (*Bromus tectorum*), with red three-awn (*Aristida longiseta*), and bluebunch wheatgrass (*Pseudoregneria spicatus*) also present. The Upper MacIntyre site is dominated by bluebunch wheatgrass, with Sandberg bluegrass, needle-and-thread grass, and cheatgrass also occurring. This site has more exposed ground and rock than Lower MacIntyre, and is slightly moister due to the influence of runoff from an adjacent cliff. Both sites are on the east side of Vaseux Lake, with a slight slope and a western aspect. The MacIntyre sites are not grazed by cattle but are visited occasionally by California bighorn sheep (*Ovis canadensis californiana*) and white-tailed deer (*Odocoileus virginianus*).

Site 3, Anarchist Mountain (49° 0'52'' N, 119° 17'0'' W, elevation 1120 m), is 100 m north along Sidley Mountain Road off Highway 3, 19 km east of Osoyoos, B.C., near the summit of Anarchist Mountain. The site is at the higher altitudinal boundary of the Ponderosa Pine biogeoclimatic zone (Meidinger and Pojar 1991). The site is relatively flat, with a slight southern aspect, and is dominated by diffuse knapweed and cheatgrass, with many patches of bare ground. The site is grazed occasionally by white-tailed deer throughout the year and by cattle every autumn. Young Ponderosa pine trees (*Pinus ponderosa*) have been cut from this site.

Biological control agents were rare at the MacIntyre sites following a wildfire in 2003. *Larinus minutus* were also rare at Anarchist Mountain.

### **Insect cages and rain shelters**

At each site, twenty replicate insect cages were placed above areas with a relatively uniform knapweed density of approximately four bolting stems per plot. This could consist of four separate plants each with one bolting stem, two plants with two bolting stems, or other variations thereof. In a few rare cases a bolt was removed by cutting with a knife to meet the four bolt requirement. Insect cages were constructed of 1.2 cm diameter white polyvinyl chloride (PVC) irrigation pipe cut to size and secured with plastic corners to make 50 cm x 50 cm x 78 cm frames. Frames were anchored with stainless steel wire to four 30 cm metal spikes in the ground and covered by grey fiberglass window insect netting (Saint-Gobain Technical Fabrics, UPC 0-55709). Insect netting was covered at the base with Scotch® duct tape and was secured firmly to the ground with nails. Cages were removed at the end of September once beetles had entered the ground for winter diapause.

Knapweed is primarily pollinated by bees, which were excluded by the insect netting. Thus, flowers in cages were hand-pollinated on two separate occasions between July 27 and August 4, 2006. Insect cage netting was carefully removed and each flower was lightly brushed with a paintbrush from other flowers either inside or near the cage.

All insect cages were covered by rain shelters regardless of watering treatment to control for any possible temperature effects. Wooden T-frames, constructed from 30 cm x 60 cm wood strips, were 120 cm high and extended beyond the cages by 30 cm on either side. Each side of the T-frame was secured to a 60 cm long base, which was secured to the ground with a 30 cm spike. A 120 cm x 210 cm sheet of Dura-film® Super 4 (152 µm) plastic greenhouse poly film (AT Plastics, Edmonton, Canada) was draped over the T-frame and secured with technical tape. The poly film was selected for its tear-resistant properties, ability to withstand adverse rain and wind conditions, high

light transmission (91%), and resistance to UV degradation. Each corner of the poly film was reinforced with technical tape and metal grommets, and was anchored with string to a 30 cm metal spike in the ground nearby (Figure 2.1). Structures were secure and only once, following a severe thunderstorm, were repairs required for three of the sixty rain shelters. Rain shelters were erected from May 23-26, 2006, and dismantled in the last week of August, 2006.

## Treatments

Each insect cage covering the knapweed was randomly assigned to one of four possible treatment combinations in a 2 x 2 factorial arrangement comprised of  $\pm L. minutus$  and  $\pm$  water. There were thus five replicates of each treatment at each field site.

*Larinus minutus* were collected from two nearby areas with high density: 1 km along MacIntyre Road (49° 17'47'' N, 119° 31'32'' W, elevation 388m), and Three Gates Farm, 1km along White Lake Road, 10 km south of Penticton, B.C. (49° 22'16'' N, 119° 37'29'' W, elevation 510m). From June 3-9, 2006, beetles were collected from these sites and added to *L. minutus* cages to a total of 40 beetles per cage (10 *L. minutus* for each knapweed stem). Cages were inspected on a minimum weekly basis and any *L. minutus* found in beetle exclusion cages were removed.

Water addition cages received 9 L of water once a week for 10 weeks, which approximated the maximum weekly precipitation recorded historically for the area. Water was added early in the morning in four batches of 2.25 L, sprinkled from a standard watering can. By applying the water in four consecutive batches the soil water tension allowed the water to seep into the soil. Watering took approximately two hours at each site. Lower and Upper MacIntyre cages were watered from June 8 to July 27 inclusive, while cages at the Anarchist Mountain site were watered from June 14 to August 2 inclusive. Soil moisture was measured to quantify the difference in conditions for knapweed plants between the watered and unwatered treatment groups. Measurements were taken once a week, two days after watering the MacIntyre sites and

three days after watering the Anarchist Mountain site. I used the time domain reflectance method (Topp, Davis & Annan 1980) to measure water content to a depth of 20 cm. Two 20 cm long stainless steel metal probes were installed in the ground within each of three, randomly-selected plots in each watering treatment group (watered or not watered). I also recorded the ambient soil moisture at the site at three arbitrarily selected areas in the vicinity of the cages. Monthly precipitation data and historical 30 year normals were obtained from Environment Canada records for the nearest weather station at Penticton, British Columbia.

### **Response measurements**

In August 2006 and May 2007, I recorded the number of bolting plants, the number of rosettes (defined as having five or more leaves), and the number of seedlings (4 or fewer leaves). From the beginning of July to the beginning of August 2006, I recorded the cumulative number of flowers per plot on a weekly basis. I recorded the height of each bolting knapweed stem at the conclusion of the experiment, in August 2006.

I recorded the number of beetles observed in cages on a weekly basis throughout the experiment. I estimated *L. minutus* herbivory in August 2006 as the percentage of the knapweed stem damaged by insect feeding.

In September 2006, I removed all bolting plants from the insect cages. An arbitrary sample of three branches from each plant stem was removed and stored at 4 °C until seedheads could be examined. All the fully developed seedheads in each sample were removed and counted. I examined a subsample of up to 25 developed seedheads from each and recorded the presence of *L. minutus* (as indicated by either an adult beetle about to emerge or frass and the absence of all seeds) and the number of seeds. The number of seeds or *L. minutus* larvae per 100 seedheads for each plot was thus

determined by adding the four totals or proportional totals from each sample of 25 seedheads.

## **Data analysis**

### *Effect of watering on diffuse knapweed*

Using only data from plots where no beetles were added, I tested for the interaction of watering and site on plant height, number of seeds per seedhead, numbers of flowers per plot in week 9, and proportion of rosettes bolting the following spring using a two-way Analysis of Variance (ANOVA) with watering and site as fixed factors. Knapweed plant population data were square root transformed for normalization and proportional data were arc sine square root transformed before performing the ANOVA. I also analyzed the full set of data from all treatment plots, including those where beetles were added with a three-way, watering x *Larinus* x site ANOVA to test for the interaction of water and site on plant response variables (JMP Version 4.0.4).

### *Effect of watering on *Larinus minutus**

Using only data from plots where beetles were added, I tested for the interaction of watering and site on the average number of *L. minutus* observed in plots on a weekly basis, the percent stem grazed by the end of the experiment in August 2006, and the number of *L. minutus* per 100 seedheads using a two-way ANOVA with watering and site as fixed factors. Percent stem grazed data were arc sine square root transformed before performing ANOVA. I also used data from all treatment plots, including those where beetles were not added, using a three-way watering x *Larinus* x site ANOVA to test for the interaction of water and site on *Larinus* response variables.

*Effect of *Larinus minutus* and watering on diffuse knapweed*

I tested for interaction of *L. minutus* and watering on plant height, the number of seeds per seedhead, the number of flowers in week 9, and the proportion of rosettes bolting the following spring using a three-way ANOVA with watering, site, and beetles as fixed factors.

*Soil moisture*

I compared soil moisture measurements from dry and watered plots with a Student's t-test, and tested for differences among these treatments at each site during the first four weeks of the experiment, during the latter five weeks of the experiment, and during the entire experiment.



## Results

I found two major effects. First, *Larinus minutus* significantly reduced seed production in both watered and unwatered plots ( $F_{1,48} = 26$ ,  $p < 0.01$ ; Table 2.1, Figure 2.2). This demonstrates that *L. minutus* were effective under both dry and wet conditions.

There was a trend towards greater seed reduction in unwatered plots (60% reduction) than in watered plots (33% reduction), but the difference was not statistically significant, and requires further experimentation. Seed production was very variable within treatment groups and there was no direct effect of watering on seed production. Overall, seed production was highest at the Anarchist site.

The second major effect was the interaction between watering and *L. minutus* addition on the number of flowers observed per plot in week 9 of the experiment ( $F_{2,48} = 4.4$ ,  $p = 0.04$ , Table 2.1). In unwatered plots, the addition of *L. minutus* beetles consistently reduced the number of flowers that had developed by week 9, while this was not the case in watered plots (Figure 2.2). Flowering at the Anarchist Mountain site was delayed by at least two weeks in comparison to the MacIntyre sites (Figure 2.3).

The average height of plant bolts did not differ between *L. minutus* treatments, watering treatments, or sites, and there were no interactions. There was also no effect of *L. minutus*, watering, and no interaction on the percent of rosettes that bolted in Spring 2007 although the rate of bolting tended to be lower in dry plots with beetles (Figure 2.2, Table 2.1).

## Soil Moisture

Soil moisture was higher in the watered treatments ( $15.1 \pm 0.5$  SE percent moisture) than in unwatered treatments ( $10.2 \pm 0.6$  SE percent moisture) ( $p < 0.01$ ). Soil moisture measured for reference purposes outside the rain shelters was  $11.4 \pm 0.6$  SE percent moisture. Overall, moisture levels were highest at the higher altitude Anarchist Mountain site (Figure 2.4). A consistent difference in soil moisture was maintained throughout the course of the experiment at the Upper MacIntyre site, while at the Lower MacIntyre and Anarchist Mountain sites, soil moisture differences were maintained during the latter half of the experiment only (Figure 2.4).

The year of the experiments, 2006, was much wetter than average: April – July precipitation was 204 mm, almost double the 29 year normal of 131 mm (Figure 2.5). The majority of precipitation occurred in the early spring (April – May), before most of the *L. minutus* beetles had emerged and rain shelters had been erected.

## Direct effect of watering on *Larinus minutus*

Although 40 beetles were added to each cage, an average of only  $14 \pm 1$  *L. minutus* were observed in beetle addition cages during weekly counts. Fewer beetles were observed at the Anarchist site than at the MacIntyre sites ( $F_{2,24} = 26$ ,  $p < 0.01$ ; Figure 2.6).

On average, only  $1 \pm 1$  beetles were observed in “no beetle” treatment cages during weekly counts, and efforts were made to ensure that any *L. minutus* found were removed. During the experiment an average total of  $4 \pm 1$  were removed from each of the beetle exclusion treatment cages.

Two-thirds of the knapweed stem surface was grazed by *L. minutus* in beetle addition plots, regardless of watering treatment. Stem grazing was higher at the MacIntyre sites than at the Anarchist Mountain site ( $F_{2,24} = 26, p < 0.01$ ; Figure 2.6). A very small amount of stem grazing occurred in the beetle exclusion cages (average of  $3 \pm 1$  percent stem grazed).

Approximately two-thirds of seedheads at the Lower MacIntyre and Upper MacIntyre sites contained *L. minutus*, while fewer (only half) of seedheads at Anarchist Mountain contained beetles ( $F_{2,24} = 5.6, p < 0.01$ ; Figure 2.6).

## **Discussion**

Here I have demonstrated that *L. minutus* is effective in reducing the number of diffuse knapweed seeds under both dry and watered conditions.

### **Potential influence of drought on seed reduction**

Overall, seed production was very variable. A trend towards greater seed reduction in unwatered plots occurred however. This could indicate that while *L. minutus* is an effective agent under both dry and watered conditions, drier conditions could potentially increase the impact of this insect. Further experimentation is required to confirm this trend.

While I found no statistical interaction of watering and *L. minutus* on diffuse knapweed seed production, plant height, and bolting rate, an exception was the interaction of *L. minutus* and watering on knapweed flower numbers per plot observed during the experiment.

### **Effect of drought and herbivory on flower numbers per plot**

While in watered plots *L. minutus* herbivory did not affect the numbers of flowers observed per plot, in unwatered plots *L. minutus* herbivory resulted in consistently fewer flowers observed by week 9 of the experiment. The reduction in flower numbers could indicate that fewer flowers are produced per plant overall, or could indicate a delay in flowering. These two possibilities could only be distinguished if the total number of flowers ultimately produced was measured.

If fewer flowers are being produced, then a reduction in seed numbers would result. If there is a delay in flowering, this could also result in potential negative fitness consequences for the plants if it influenced pollination success. Delayed flowering is a strategy that allows plants to maximize available resources for reproduction (Springer &

Ward 2007). In this case, the plant may “buy time” to recover from drought and herbivory before devoting resources to reproduction.

Drought conditions can influence the physiology of plants in several ways. In the short-term moisture stress may cause plants to lose turgor, leaf water potential to decrease, and stomates will close more readily. In the long-term, downregulation of metabolic processes under drought conditions results in an inhibition of photosynthesis (Flexas & Medrano 2002). Proteins are eventually hydrolyzed, and levels of amino acids increase, with a resultant decrease in the carbon to nitrogen (C:N) ratio in plant tissues (Brodbeck & Strong 1987).

Herbivory can further depress the C:N ratio if it is associated with an increase in available nitrogen in plant tissue (McNaughton 1979; White 1969). Because flowering induction is associated with carbohydrate-regulated gene expression (Rolland, Baena-Gonzalez & Sheen 2006), the additive effect of drought and herbivory results in a significant drop in the C:N ratio. Without the excess carbohydrate required for signaling (Corbesier, Bernier & Perilleux 2002), flowering will be delayed and plants will devote their resources to recovery from the combined stresses of drought and herbivory.

Delayed flowering can have serious implications for plant fitness, since it can potentially affect subsequent interaction with pollinators (Memmott et al. 2007). Plants that delay flowering may compensate for insect attack but run the risk of missing the key window for pollination to occur. Juenger and Bergelson (1997) reported that plants subjected to simulated herbivory (clipping) were only able to compensate for damage upon receiving supplemental hand pollination. Totland (1997) found that later flowering plants produced fewer and lighter seeds than plants that flowered one week earlier.

A further complication can occur if rising temperature accelerates insect pollinator development, as predicted by climate change studies (Bale et al. 2002) and long-term observations of honeybee phenology (Gordo & Sanz 2006). In this diffuse knapweed-*L. minutus* study, because plants were hand-pollinated, any potential

consequences for the subsequent pollinator-plant interaction were not observed. It is possible however that under natural conditions, knapweed plants with delayed flowering could receive less than adequate pollination, with the resultant fitness consequence of reduced seed production.

### **Synchrony and biological control systems**

Variation in the synchrony of flower development and *L. minutus* oviposition associated with drought and stem damage could also have an impact in this system. Delayed flowering might allow knapweed plants to “escape” the peak oviposition period for *L. minutus*. I found no evidence for this “escape” however as *L. minutus* numbers did not differ between watered and unwatered treatments in the next generation. Given sufficient genetic variation, strong selective pressure would likely maintain the synchrony of *L. minutus* and knapweed flowering even in the face of climate change (van Asch et al. 2007).

Kingsolver (1996) demonstrated that insects with small thermal ranges may evolve responses more readily than climate generalists. Adaptation has already been observed in *Chrysolina* beetles, originating from Australia but raised in California before their eventual release in British Columbia for the control of St. John’s wort (Peschken 1972).

### **Response of *Larinus minutus* to changes in soil moisture**

Watering had no apparent effect on *L. minutus* activity, as measured by stem grazing, beetle counts, and next generation beetles. These results suggest that *L. minutus* beetles are robust to changes in moisture conditions.

The plant stress hypothesis (White 1969, 1984) predicts that increased insect herbivore performance and population outbreaks during drought conditions can be

attributed to changes in available foliar nitrogen. Under water deficit conditions, the ability of the plant to synthesize proteins is impaired, resulting in an increase in available nitrogen in plant tissue (Hsiao 1973). Because herbivorous insects are generally nitrogen limited (McNeill & Southwood 1978), the availability of such nitrogen can be a key determinant of herbivore populations (Sinclair 1974; White 1978). It therefore follows that an increase in the available nitrogen would result in an increase in herbivore performance.

Support for the plant stress hypothesis from other experimental studies of drought effects is mixed (review in Huberty and Denno 2004). Larsson (1989) found that insect performance response to plant stress is complex, and depends on feeding guild, while Price (1991) proposed the plant vigour hypothesis, that herbivorous insects feed preferentially on more vigorous plants or plant modules. Huberty and Denno (2004) proposed the “pulsed stress hypothesis”: drought stress alternates with periods of increased water availability (and therefore turgor recovery), thus providing insects with the opportunity to access the increased nitrogen in plant tissues.

It is often not possible to determine if correlations between stressed plants and insect outbreaks are due to changes in plant suitability for insects (Myers 1988). Drought stress is often accompanied by warmer than usual temperatures, that enhance insect growth and survival (Koricheva, Larsson & Haukioja 1998; Larsson 1989).

Biological control insects are specialist feeders, and are dependent on their host plant for all of their nutrient requirements. Therefore, like other specialist insects, they are more likely to have evolved strategies to compensate for fluctuating levels of nutrients in their host plants (McNeill & Southwood 1978; Prestidge & McNeill 1983). Therefore, changes in nitrogen levels may not elicit a response in these insects. Recent work supports this concept: generalist insect herbivores of montane *Asteraceae* are more affected by climatic conditions along an altitudinal gradient than specialist herbivores (Scheidel, Rohl & Bruelheide 2003).

## Site differences

While plant height was consistent among all three sites, seed production was substantially greater at the Anarchist site. The Anarchist site is at a higher elevation than the other two sites, and precipitation is greater and more frequent. The site is subject to two key disturbance regimes: cattle grazing in the fall, and small-scale disturbances by pocket gophers. Knapweed density is likely higher due to occasional increases in gross resource supply (e.g. precipitation) and decreased uptake by plants (due to cattle grazing and small disturbances caused by gopher activity). This is consistent with the fluctuating resource availability theory of invasibility proposed by Davis et al. (2000).

The Anarchist site also differed from the MacIntyre sites in terms of *L. minutus* activity and survival. Fewer *L. minutus* apparently survived the introduction to cages at Anarchist, with approximately 25% fewer insects observed during weekly counts. Stem and seed herbivory were also lower at the Anarchist site. It is possible that this was due to the colder temperatures, that would reduce insect activity (Bale et al. 2002). Although *L. minutus* is found throughout Eurasia, beetles introduced in British Columbia are imported from Greece and are therefore adapted to a warmer climate (Groppe 1990). These results suggest that it may be prudent to introduce a number of biotypes in order to have sufficient genetic variability to achieve optimum success in new habitats of introduction, to enable dispersal to new habitats, and to provide sufficient genetic material for populations to adapt to future changes in climate. Population studies on a broad regional scale in the native range will be helpful in selecting populations for potential screening. However, host plant specificity testing of the different biotypes is important, because disparate populations can show apparent specificity while the species as a whole can use hosts over a wider range (Klein & Seitz 1994; Sheppard, van Klinken & Heard 2005).



## **Timing of drought and herbivory and design constraints of the experiment**

The rain shelters were effective in controlling for moisture due to natural precipitation. Early spring precipitation in April and May, prior to erection of the rain shelters, however was 85% higher than the 30 year normal (Figure 2.5), and soil moisture was retained even in unwatered treatment plots at two sites for 3-4 weeks after shelters were erected (Figure 2.4). Plants achieved a great deal of growth prior to both the emergence and introduction of the *L. minutus* beetles and the initiation of the watering treatments. Knapweed plant fitness may be substantially influenced by early spring conditions when bolting is initiated, or even by weather conditions in the previous year as a rosette. To adequately test the interactions between precipitation and the impacts of *L. minutus*, rain shelters would need to be erected in late March or early April before the beginning of plant bolting. Early erection of the rain shelters would also ensure that differences in soil moisture are maintained throughout the window of interaction between *L. minutus* and knapweed. The most consistent differences in soil moisture were observed during the latter half of the experiment, from the fifth week of the experiment onwards as the onset of flowering began. This may explain why I found an interaction of herbivory and soil moisture on flower numbers during week 9 of the experiment but not on plant height.

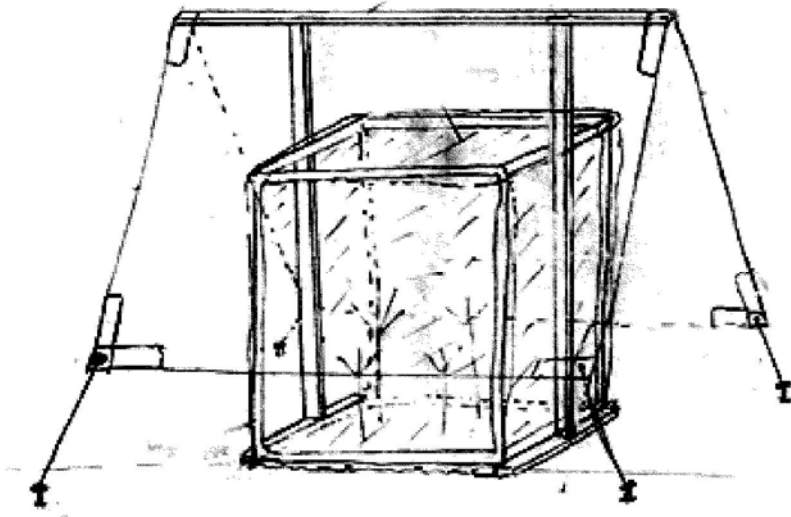
## Conclusion

This study examined how climate change, particularly a change in precipitation, might affect the efficacy of a biological control system. I have demonstrated that *L. minutus* are capable of reducing diffuse knapweed seed production regardless of soil moisture conditions and thus their impacts will not be limited to only certain conditions. Therefore, biological control of diffuse knapweed by *L. minutus*, while possibly more effective under dry conditions, is generally robust to changes in precipitation.

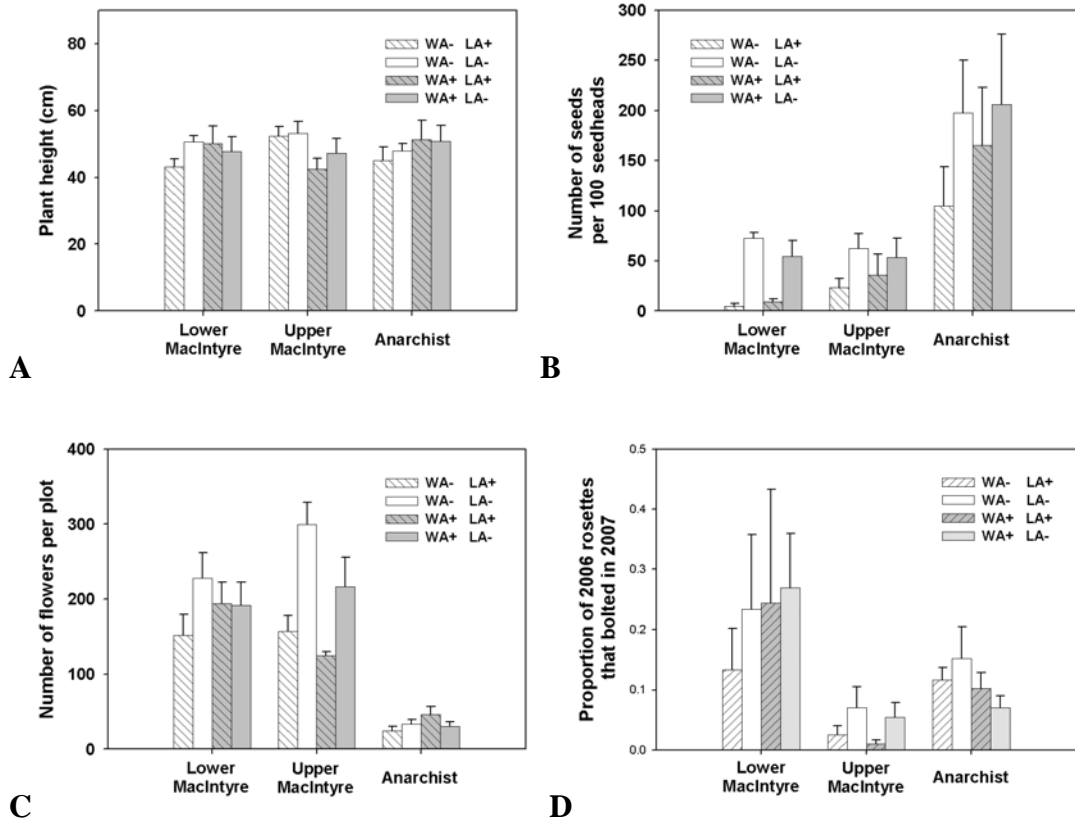
Increased variability in weather patterns and other disturbance regimes may make ecosystems more susceptible to the invasion of non-native species. Biological control agents as species-specific feeders are likely to tolerate fluctuations in nutrient and water conditions of target host plants. With increasingly variable weather patterns as predicted under global climate change these results serve to underscore the importance of biological control as a tool for invasive species management.

**Table 2.1.** Effects of *Larinus minutus*, watering, and site on diffuse knapweed height, seed production, cumulative number of flowers per plot in week 9 of the experiment, and bolting of rosette plants.

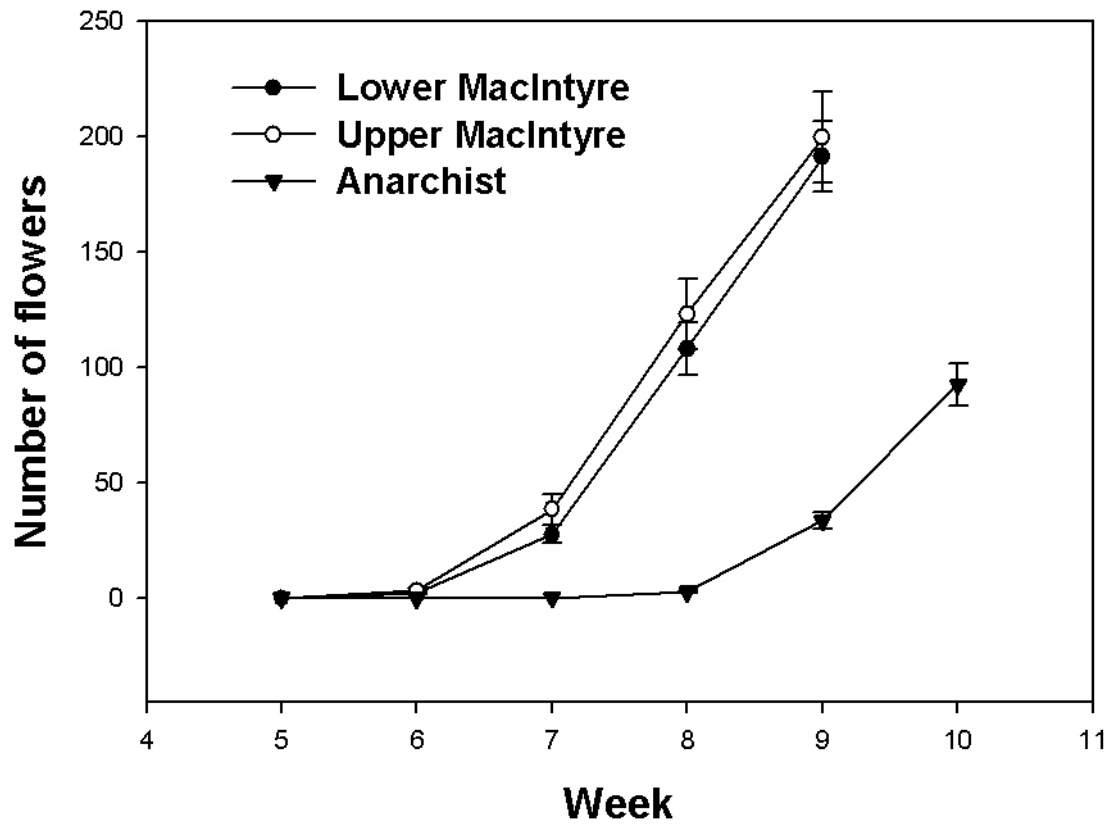
	Plant height		Number of seeds per seedhead		Cumulative number of flowers per plot in week 9		Proportion rosettes bolting the following spring	
	df	P value	df	P value	df	P value	df	P value
<b>Water</b>	1,48	0.86	1,48	0.57	1,48	0.49	1,48	0.89
<b>Site</b>	2,48	0.94	2,48	< <b>0.01</b>	2,48	< <b>0.01</b>	2,48	< <b>0.01</b>
<i>Larinus</i>	1,48	0.34	1,48	< <b>0.01</b>	1,48	< <b>0.01</b>	1,48	0.41
<b>Water * <i>Larinus</i></b>	1,48	0.50	1,48	0.21	1,48	<b>0.042</b>	1,48	0.65
<b>Water * Site* <i>Larinus</i></b>	2,48	0.47	2,48	0.59	2,48	0.86	2,48	0.92



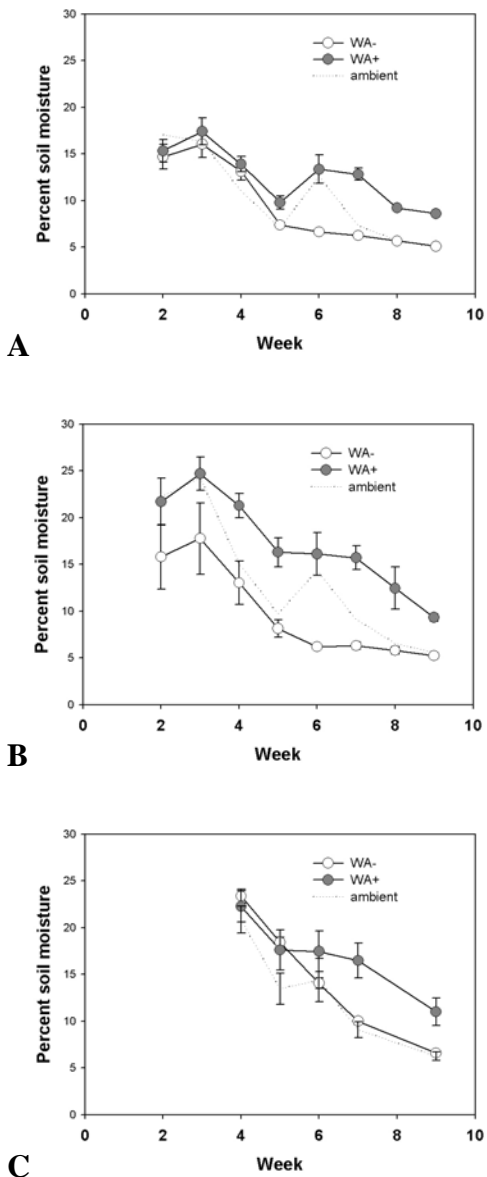
**Figure 2.1.** Field experiment design: rain shelter over insect cage



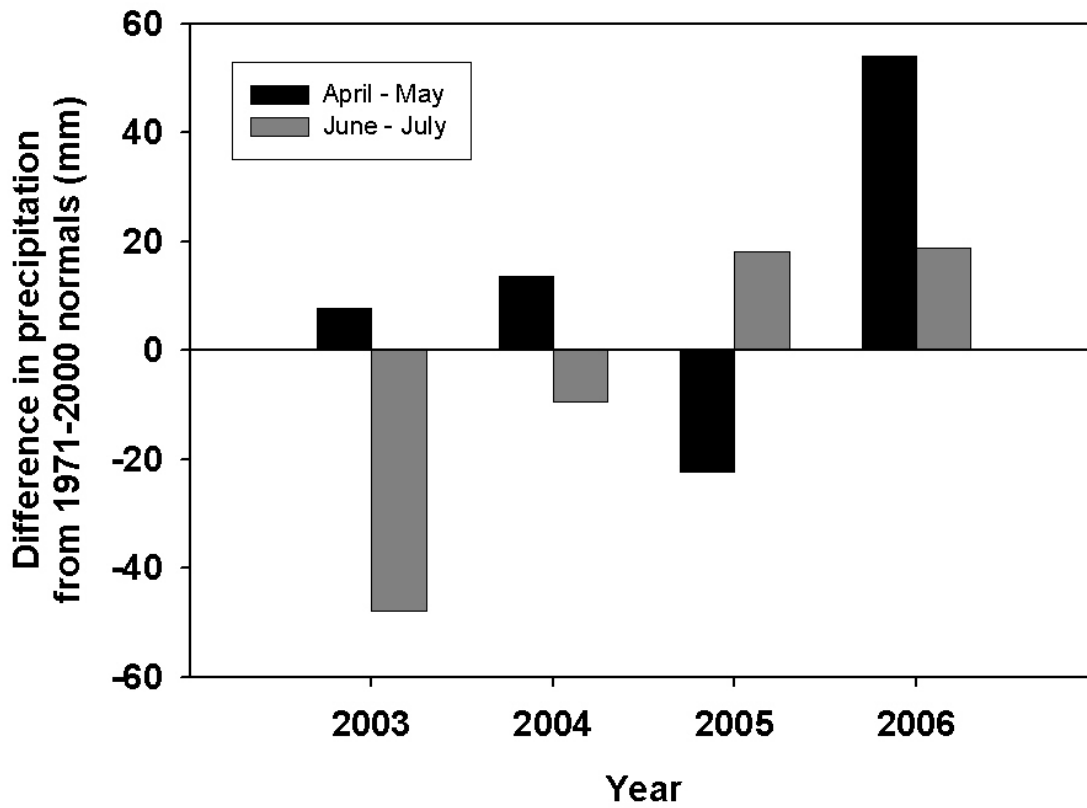
**Figure 2.2.** Diffuse knapweed response to watering (WA) and *Larinus minutus* (LA) treatments: **A**) Plant height ( $\pm$  SE) in August at the conclusion of the experiment; **B**) Number of seeds per 100 seedheads ( $\pm$  SE); **C**) Cumulative number of flowers per plot ( $\pm$  SE) by week 9 of the experiment, the last week of July; **D**) Proportion ( $\pm$  SE) of rosettes in August 2006 that bolted in May 2007.



**Figure 2.3.** Mean number of flowers ( $\pm$  SE) observed per plot over the course of the experiment. The Anarchist site was approximately two weeks behind the MacIntyre sites.

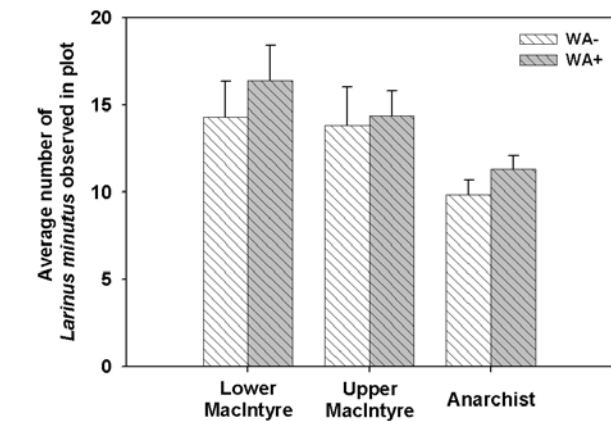


**Figure 2.4.** Mean ( $\pm$  SE) percent soil moisture of watered (WA+) and unwatered (WA-) treatments, and ambient moisture outside rain shelters. Overall, there was a difference between watered and unwatered plots ( $p < 0.01$ ); **A**) Lower MacIntyre: weeks 2-4  $p = 0.48$ ; weeks 5-9  $p < 0.01$ ; **B**) Upper MacIntyre: weeks 2-4  $p < 0.01$ ; weeks 5-9  $p < 0.01$ ; and **C**) Anarchist Mountain: week 4  $p = 0.57$ ; weeks 5-9  $p < 0.01$ . Soil moisture was not measured during weeks 2, 3, and 8 at the Anarchist site.

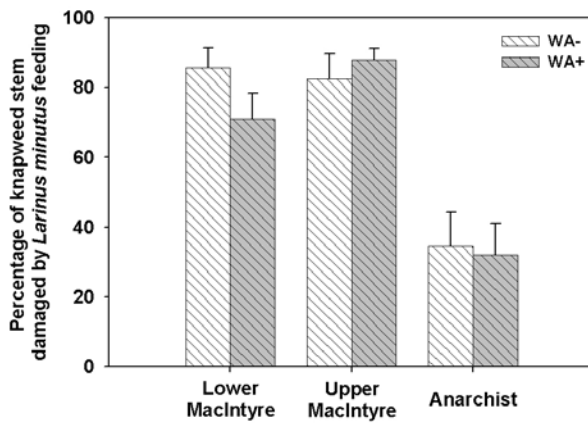


**Figure 2.5.** Difference in precipitation (in mm) for 2003-2006 years from 1971-2000 Environment Canada normals for total spring (April-May) and early summer (June-July) precipitation recorded at Pentiction, British Columbia weather station.

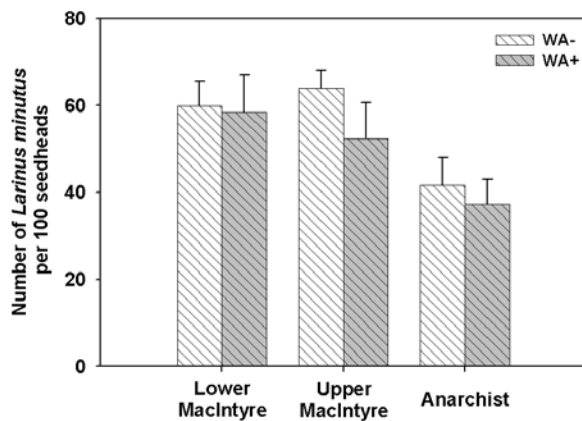




A



B



C

**Figure 2.6.** *Larinus minutus* response to watering (WA) treatments: **A)** Average number of *L. minutus* observed in plots; **B)** Average percentage of knapweed stem damaged by *L. minutus* feeding, visually estimated in August 2006; **C)** Number of *L. minutus* per 100 seedheads. Error bars are standard error.



**Figure 2.7.** Rain shelter experimental setup (Anarchist site)



**Figure 2.8.** *Larinus minutus* feeding damage on diffuse knapweed stem



**Figure 2.9.** *Larinus minutus* emerging from diffuse knapweed seedhead

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## Chapter 3: Species pairs for the biological control of weeds: advantageous or unnecessary?<sup>2</sup>

### Introduction

The issue of single vs. multiple agent releases in the biological control of weeds has been a subject of much debate. Some have asserted that choosing the most effective agents will reduce the potential for undesirable nontarget effects (Louda et al. 2003). Denoth et al. (2002) reviewed cases of successful biological control of weeds and found that a majority of successes are attributed to a single agent. They suggest that additional agents should be introduced only if reductions in plant density are not being achieved by the initially introduced agent(s). Where two or more agents are released simultaneously, they should be released in geographically distinct areas where the differential success of the agents can be assessed.

The success of biological control agents in reducing target plant density depends on factors such as climate, target weed phenology, nutrient conditions, dispersal ability, fecundity, and type and level of damage to key life stages of the plant. In some cases two species in the same genus have been introduced even though they are very similar in their interactions with their host plants. This has sometimes been by accident, such as the case of the two species of *Urophora* and the mixture of the two species of *Galerucella*, and sometimes on purpose from different habitats. When the species are difficult to distinguish morphologically, considerable confusion can occur over the impacts, distributions, and coexistence of each. This is currently the situation with *Larinus minutus* Gyllenhal. and *L. obtusus* Gyllenhal. introduced on knapweeds in North America (Harris 2005).

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The competitive exclusion principle predicts that two very similar sympatric species cannot occupy the same niche and therefore, for coexistence, even very similar species must have some differences in niche requirements (Hardin 1960). It is of interest therefore to know if differences between congeneric biological control agents contribute to their successful control of target weeds. For example, if congeneric species act in a cooperative and complementary fashion to reduce plant densities, their introductions would be warranted. The greatest advantage of congeneric biological control agents occurs if differential climatic tolerances result in effective control of the host plant across different biomes.

Here we address two questions in regard to congeneric releases: (1) is there evidence that both species are necessary for successful biological control; and (2) do congeneric species exploit different climatic regions and thus broaden the geographic effectiveness of biological control?

## Methods

We restricted our analysis to agents released to control weeds in North America, based on studies reported in Coombs et al. (2004) and Mason and Huber (2002). Of the 25 weed control programmes reported in Coombs et al. (2004), 19 (76%) involved the release of more than one species of biological control agent insect, and 10 (40%) involved the release of congeneric species of agents.

For each case of congeneric biological control agent releases, we scoured the literature, searching Web of Science using agent species names as the search terms, paying particular attention to papers where the two species had been compared. We also searched for papers in the literature cited sections of each paper to find additional information.

We excluded from further analysis species pairs or combinations for which clear differences existed between the species in terms of target weeds. We also excluded species that had not yet been established, or for which insufficient post-release information was available for appropriate comparisons. In addition, we excluded the *Aphthona* flea beetle species released for control of leafy spurge, *Euphorbia esula* L. Five species, *A. czwalinae* (Weise), *A. lacertosa* Rosenhauer, *A. cyparissiae* (Koch), *A. flava* Guillebeau, and *A. nigriscutis* Foudras have been released in the U.S. and Canada, and a sixth species, *A. abdominalis* Duftschmidt, has been released in the U.S., but failed to establish (Hansen et al. 2004). The five species were introduced because of differences in habitat preferences, but control of *E. esula* in shrubby riparian areas remains a challenge (Bourchier et al. 2002). Due to the large number of different species released, their recent releases, and the lack of comparative data between all combinations, we did not consider this group of congeneric species. We also did not consider agents currently under consideration for release. The four species pairs discussed in detail below represent a range of habitat (grassland, riparian, aquatic) and target weed types and have (1) established, (2) reached substantial populations, (3) been studied in their non-native range, and (4) been compared in the literature.

## Results

### **St. John's wort, *Hypericum perforatum* L.: *Chrysolina hyperici* (Forster) and *Chrysolina quadrigemina* (Suffrian)**

The defoliating beetles *Chrysolina hyperici* and *C. quadrigemina* comprise two of the five species of biological control insects established in North America for control of the rangeland weed St. John's wort (*Hypericum perforatum*). These two beetles represent some of the earliest attempts at biological control, having been introduced in 1945 and 1946 respectively to the United States (Piper 2004) and in 1951 to Canada (Harris 1962).

*Chrysolina hyperici* originates from northern and central Europe and western Asia. Eggs are laid in the fall (or in the spring in the colder continental interior) (Piper 2004). Larvae hatch and feed on leaf buds and leaves and pupate in the soil. Adults emerge in the spring, feed, and then enter the soil for summer diapause before emerging in the fall to mate and lay eggs.

The native range of *C. quadrigemina* extends further south from Denmark to North Africa, and this species prefers warmer, drier areas. Both *Chrysolina* species are univoltine, and have similar life cycles but slightly different phenologies.

Field and laboratory studies in New Zealand show that for both *Chrysolina* species, the termination of summer diapause is triggered by shortening daylength. However, *C. quadrigemina* terminates summer diapause approximately three to four weeks earlier, at a daylength of approximately 13.5 h compared to 12.5 h for *C. hyperici*. *Chrysolina quadrigemina* females reach sexual maturity more quickly and therefore oviposit earlier. In areas with mild winters *C. quadrigemina* is considered to be the superior agent because the larvae feed on plants for a longer time than *C. hyperici* larvae (Schops, Syrett & Emberson 1996).

Early studies in British Columbia by Harris (1962) suggested that *C. hyperici* could be the superior agent in areas with early frosts. Because *C. hyperici* oviposition occurs on average a month later than that of *C. quadrigemina*, many of the eggs do not hatch until the following spring. Therefore, a greater portion of the next generation overwinters in the more resistant egg stage, thus escaping winter larval mortality.

In a subsequent assessment, Harris et al. (1969) found *C. quadrigemina* to dominate in dry subhumid sites, particularly those with Ponderosa pine (*Pinus ponderosa* Douglas ex Lawson et. C. Lawson), while *C. hyperici* was most effective in moist subhumid sites where Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) was present. Both species of beetle were successful in reducing *H. perforatum* populations.

Peschken (1972) compared *C. quadrigemina* from British Columbia with *C. quadrigemina* from California (the original source of the British Columbia [B.C.] introductions two decades previously), to determine if post-colonization adaptation had occurred. The B.C. beetles laid a larger number of eggs per female, and this could increase the number of beetles surviving the harsher winters to the next generation. Also, the B.C. beetles demonstrated a greater tendency to seek shelter under cold temperature conditions. Although *C. hyperici* appeared initially to be the more effective agent in northern latitudes, its intolerance of dry conditions may limit its overall effectiveness.

Campbell and McCaffrey (1991) similarly concluded that in Northern Idaho, *C. hyperici* beetles attack plants in more mesic forested areas while *C. quadrigemina* dominates at grassland sites where the target weed *H. perforatum* most commonly occurs.

Overall, it is clear that worldwide, *C. quadrigemina* is responsible for the majority of the reduction in *H. perforatum* populations; however, in areas with very cold winters or more mesic sites at higher elevation, *C. hyperici* offers good complementary control (Schops 1996; Jensen, Harris & Sampson 2002).

Such prior knowledge of differences in phenology and cold hardiness could inform future agent introduction and redistribution efforts in other biological control programmes.

**Diffuse knapweed, *Centaurea diffusa* Lamark, spotted knapweed, *Centaurea stoebe* ssp. *micranthos* (S.G. Gmelin ex Gugler) Hayek and meadow knapweed, *Centaurea pratensis* Thuill.: *Urophora affinis* Frfld. and *Urophora quadrifasciata* (Meig.)**

The gallfly *Urophora affinis*, was released in Canada in 1970 and in the U.S. in 1973 for control of diffuse and spotted knapweed in the genus *Centaurea* (Harris 1980a) (Story, Nowierski & Boggs 1987). *Urophora affinis* oviposits in immature flower heads, inducing the formation of a woody gall in the receptacle, and reduces knapweed seed production (Harris 1980b; Shorthouse 1989). Another gall fly, *U. quadrifasciata*, was accidentally released in 1973. *U. quadrifasciata* oviposits in slightly larger, more mature flower heads, induces the plant to form a thin, papery gall in the ovary, and also reduces seed production (Harris 1980b).

*Urophora affinis* is primarily univoltine whereas *U. quadrifasciata* is partially bivoltine. In western Montana, the peaks of first generation emergence of the two species occur approximately one week apart: 25 June for *U. affinis* and 2 July for *U. quadrifasciata* (Story, Boggs & Good 1992). At the time of fly emergence, the majority of knapweed flower heads are at the most suitable stage for *U. affinis*. Attack by *U. affinis* stunts the growth of the remaining heads so that many do not reach the size acceptable to *U. quadrifasciata* (Berube 1980), and thus they must disperse to find sites with capitula suitable for oviposition for the second generation. This could account for the broader distribution of *U. quadrifasciata* in Canada and the U.S. (Story & Coombs 2004a, 2004b) despite *U. affinis* being less active flier (Roitberg 1988). In a survey in Montana, *U. quadrifasciata* occurred at almost all sites examined as compared to *U. affinis* that was found at approximately half of the sites (Story, Nowierski & Boggs 1987).

Surveys of original release sites in British Columbia have revealed that *U. affinis* is most often the dominant species (Harris 1980a; Myers unpublished data), in agreement with predictions made by Story (1992). However, because of geographic and annual variation in flower head sizes at emergence times, it is unlikely that *U. affinis* will eventually displace *U. quadrifasciata* (Berube 1980). In addition, the supercooling capacity of *U. affinis* is superior to that of *U. quadrifasciata* (Story, Good & Callan 1993), and Nowierski et al. (2000) conclude that *U. affinis* is more tolerant of cold winter temperatures. This could also help to explain the predominance of *U. affinis*, particularly at the more northerly release sites.

The exception to *U. affinis* dominance is in southwest Virginia, where surveys have shown that *U. quadrifasciata*, which was not introduced but is thought to have dispersed from releases in Maryland, now outnumbers *U. affinis*. The longer growing season in this area may favour *U. quadrifasciata*, although it does not appear to have displaced previously established *U. affinis* populations (Mays & Kok 2003).

*Urophora affinis*, although smaller than *U. quadrifasciata* (Roitberg 1988), reduces seed production by 2.4 seeds/head in comparison to 1.9 seeds/head for *U. quadrifasciata* and thus could be considered to be a more effective agent (Harris 1980a). Myers and Harris (1980) found that overall seed reduction was slightly greater when both agents were present in combination. Because *Centaurea* is not seed limited, however, these gallflies have not been successful in reducing overall weed density (Myers & Risley 2000).

**Water hyacinth, *Eichhornia crassipes* (Mart.) Solms: *Neochetina eichhorniae* Warner and *Neochetina bruchi* Hustache**

The weevil *Neochetina eichhorniae* was released in the southern United States in 1972. The adults and larvae feed on water hyacinth (*Eichhornia crassipes*) leaves, often damaging meristematic tissues, and leaving distinct scars that limit plant growth.

*Neochetina bruchi* feeds in a similar manner. Both species are multivoltine (Center 2004; DeLoach & Cordo 1976).

Both *Neochetina* species are capable of reversibly generating and degenerating flight muscles, to correspond with dispersal and reproductive phases. *N. bruchi* appear to be more sensitive to plant quality and require high nitrogen content to sustain their high fecundity (Spencer & Ksander 2004). On stressed plants, Center and Dray (1992) found more *N. bruchi* with developed flight muscles than *N. eichhorniae*. Differences in preferences for oviposition sites may decrease interspecific competition and lead to complementary effects, because *N. eichhorniae* prefers to oviposit on younger more central leaves, while *N. bruchi* prefers to oviposit on older, more outer leaves (DeLoach & Cordo, 1976).

*Neochetina bruchi* appears to be the superior agent, mainly because it has a faster population growth rate; the females lay more eggs, and the larvae develop faster. Thus, these beetles can kill plants faster than *N. eichhorniae* (DeLoach & Cordo 1976). The two species occur together throughout their native range, although *N. eichhorniae* predominates at warmer latitudes in northern Argentina, Paraguay, and Brazil. Also, *N. eichhorniae* is more tolerant of extremely high temperatures for oviposition, adult feeding, and adult and egg survival. *N. bruchi* adults, in contrast, survive better at lower temperatures (DeLoach & Cordo 1976). In Florida, *N. eichhorniae* is the more widespread agent (Center & Dray 1992; Center et al. 1999), and it is this agent that is most often credited with the control of *Eichhornia crassipes* in North America (Center 1987; Center & Durden 1986; Goyer & Stark 1984).

It is possible that *N. eichhorniae* may be more suitable at warmer latitudes, while *N. bruchi* may be more effective at cooler temperatures and under higher nutrient levels (Heard & Winterton 2000).

**Purple loosestrife, *Lythrum salicaria* L.: *Galerucella pusilla* Duftschmidt and *G. californiensis* L.**

The defoliating beetles *Galerucella pusilla* and *Galerucella californiensis* were introduced to North America as mixed releases in 1992 (Hight et al. 1995). Adults and larvae feed on the buds and leaves of purple loosestrife (*Lythrum salicaria*) killing plants or reducing their vigour in subsequent years. The two species of beetles are very similar in life history strategies and appearance and they can only be distinguished by dissection of the males. In a study of the beetles in their native range Blossey (1995) concluded that these very similar species, that make identical use of their shared food resource, are able to coexist. He proposed that coexistence could be due to differences in the competitive abilities of individuals, as proposed by Begon and Wall (1987): individual variation, rather than niche differentiation, promotes the coexistence of competing species.

In a survey of Central New York completed in 2004, ten years after the initial introduction of the beetles, Grevstad (2006) found that *G. pusilla* was generally more abundant than *G. californiensis*, but that the abundance of *G. californiensis* was increasing. Grevstad concluded, however, that the two species did not occupy identical niches, and that coexistence could be due to the greater dispersal abilities of *G. californiensis*, which allows the beetle to coexist with an almost identical competitor, *G. pusilla*.

McAvoy and Kok (2004) found that the phenologies of both species are almost identical, and are well synchronized with the host plant. One species completes egg development more slowly, while the other species has faster larval development. In contrast to *Neochetina* beetles, neither beetle species exploits the lower quality food source of older leaves.

McAvoy and Kok (2004) concluded that at higher, colder latitudes in North America, the overall better cold tolerance of *G. californiensis* makes it a superior competitor. The situation is complex however because *G. californiensis* larvae feed more at lower



temperatures, but *G. pusilla* larvae may survive better when food is limiting, because they require less food for development (McAvoy & Kok 2007).

In western Oregon and Minnesota *G. pusilla* is the dominant species (Schooler 1998; L. Skinner, pers. com.) although *G. californiensis* is more common at northern locations in Minnesota (L. Skinner, pers. com.). In Michigan, where mixtures of the two species were originally introduced, based on morphological identification only *C. californiensis* currently occur (Landis et al. 2003; D. Landis, pers. com.). In Canada beetles initially came from the mixed species stock in the United States. In 2005, it was found that in Ontario *G. californiensis* dominated sites of mixed releases that had been made in the mid-1990s (J. Corrigan, pers. com.). This is similar to observations in Michigan and Minnesota but conflict with the continued dominance of *C. pusilla* in New York. In western Canada species identifications were not confirmed but are thought to be *G. californiensis* (Denoth & Myers 2005; Lindgren, Corrigan & De Clerck-Floate 2002).

The difficulty of distinguishing the two *Galerucella* species complicates analysis but *G. californiensis* seems to be the superior agent in terms of dispersal and persistence particularly in the North. Successful biological control apparently occurs with either species alone or together.

## Discussion

Biological control is highly context dependent. Agent persistence and plant damage depend on target plant nutritional status and phenology. Also, insect agents are highly sensitive to local climatic conditions of temperature, precipitation, and humidity. With increasingly variable trends in climate due to global change, having more than one agent could serve as an insurance policy against fluctuations in survival due to environmental conditions.

The two *Chrysolina* beetle species seem to offer complementary control of St. John's wort on a continent-wide scale. Overall, *C. quadrigemina* is probably responsible for most of the control of the weed, with *C. hyperici* offering complimentary control in more mesic, higher elevation and forested sites. This pattern is similar to the latitudinal gradient in *Galerucella* spp. with control of purple loosestrife by *G. pusilla* being dominant at more southerly locations and *G. californiensis* dominant at more northerly locations. In the case of the *Urophora* gallflies, although *U. affinis* is superior in terms of seed reduction, overall seed reduction is slightly greater with the two species but the seed reduction is insufficient for successful biological control.

Caution, however, is advised. In some cases competition from an inferior agent can result in reaching suboptimal levels of control (Crowe & Bouchier 2006). Furthermore, under certain environmental conditions it may be best to introduce only one or other of the agents. For example, under high nutrient conditions, release of *N. bruchi* could result in greater control than release of *N. bruchi* in combination with *N. eichhorniae*.

Along with the risk of achieving sub-optimal control due to competition from similar species, with each new species introduction comes the risk of non-target effects on ecosystems, a subject that has received much attention of late (Cory & Myers 2000; Strong & Pemberton 2000; Louda et al. 2003). We conclude that the best strategy is careful field and laboratory pre-release experimentation in the native habitat, followed by the evaluation of replicated releases of individual species into geographically distinct

areas. Finally, we recommend that in addition to maintaining voucher specimens of insect releases, molecular tools for species identification be developed so that mixed stocks of species and strains can be identified.

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## Chapter 4: General conclusion

### Summary

One of the consequences of climate change is the possible disruption of species interactions, in particular the effects of predators on prey resulting in changes of trophic cascades. This problem is particularly relevant to biological control because successful depredation by control agents could become disrupted if climate parameters are changed. Hence, researchers have highlighted the need to integrate biological invasion and climate change biology (Hellmann et al. 2008). Climate mediates plant-insect interactions (Kingsolver 1989; Bale et al. 2002) particularly through precipitation and temperature characteristics, and consequently it could influence biological control efficacy in either positive or negative ways.

Here I have examined how climate could impact the efficacy and range of biological control agents. In the second chapter, I designed a rain shelter experiment to determine the effect of precipitation on a biological control system. Specifically, I asked if control of diffuse knapweed (*Centaurea diffusa*) by the bioagent *Larinus minutus* was more or less effective under conditions of reduced soil moisture. In the third chapter, I asked whether two congeneric species were necessary for successful biological control, and whether congeners could exploit different climatic regions and thus broaden the geographic effectiveness of biological control.

The main findings of this thesis are that:

### **Biological control of *Centaurea diffusa* by *Larinus minutus* occurs regardless of precipitation conditions**

I found that *L. minutus* reduced seed production regardless of moisture conditions, with a trend towards potentially greater reduction in drier, unwatered plots.

## **Congeneric agents can offer complementary control of target weeds in slightly different habitats**

In the cases of *Galerucella californiensis* and *G. pusilla* releases on *Lythrum salicaria*, along with *Chrysolina hyperici* and *C. quadrigemina* releases on *Hypericum perforatum*, the species pairs appear to offer complementary control of the target weeds, with both species providing good control in slightly different habitats and coexisting at some sites. In the case of the *Urophora affinis* and *U. quadrifasciata* gallflies released for *Centaurea* species, overall seed reduction is greater when both agents are present together, even though this seed reduction is not sufficient for overall control of the target weed. With respect to *Neochetina* beetles released for the control of *Eichhornia crassipes*, *N. bruchi* was the superior agent, with faster population growth and hence the ability to kill plants faster than *N. eichhorniae*. However, *N. eichhorniae* is more widespread, and is most often credited with control of *E. crassipes*, so whether the congeners broadened the geographic effectiveness of biological control was not clear. In summary, care is required to avoid mixed species introductions because interpretation of the agents' efficacy is prevented by confounding effects of the two species. In general controlled field experimentation of individual species is crucial to determine the respective species impacts. Molecular studies are recommended to prevent the accidental introduction of multiple species.

## **Suggestions for further research**

The results I have discussed lead to a number of new areas for investigation which would further our understanding of how climate affects the efficacy of biological control.

**1. Can the time scale needed to achieve acceptable control be predicted through modeling?** Here I have quantified the effect of reduced watering on the control of diffuse knapweed by *L. minutus*. It is now possible to determine how an increase in the frequency of summer drought conditions would affect the time scale needed to achieve an

acceptable level of control. This could be accomplished by building a simple balance equation model that incorporates summer precipitation conditions and their effect on seed, seedling, and rosette survival (with and without *L. minutus*). I predict that with increased frequency of drought, the knapweed seedbank could be reduced more quickly, seedling and rosette mortality could be higher, and knapweed density could decline more rapidly than under normal precipitation conditions.

**2. What is the effect of altitude on biological control?** Observational evidence suggests that *L. minutus* could be less capable of reducing diffuse knapweed densities at higher elevations. This could be due to the effect of colder temperatures, more frequent precipitation, or other factors. With a regression experiment with low, medium, and high altitude sites it would be possible to test the interaction of altitude and herbivory, and how this is mediated by altitudinal differences in plant and insect phenology. I predict that diffuse knapweed will be less susceptible to *L. minutus* attack at higher altitudes. Invasive plants could be capable of tolerating a broader range of climate than their biological control agents. *Chrysolina hyperici* and *C. quadrigemina* together appear to offer good complementary control of *Hypericum perforatum*, with *C. hyperici* more effective at higher altitudes.

**3. What is the impact of delayed flowering on subsequent interactions with pollinators and seed production?** Here I suggest that delayed flowering caused by the interaction of drought and *L. minutus* could result in a fitness consequence for diffuse knapweed plants if the majority of flowers miss the key window for pollination. To confirm this, the experiment would need to be repeated, with plots exposed to natural pollinators: cages would be removed so that natural pollinators can access the plants for a two-week period. A subsequent comparison of seed numbers and weights from each treatment group would reveal fitness consequences. I predict that delayed flowering will result in fewer seeds, and that these seeds will be less likely to germinate.

**4. How does timing of precipitation impact the attack by *L. minutus* on diffuse knapweed?** Additional study is required to determine how the timing of spring and summer precipitation influences diffuse knapweed growth and the effect of *L. minutus* attack. An experiment could be designed with rain shelters and three drought timing treatments and controls (early spring, late spring, and summer) combined with *L. minutus* addition and controls to determine how the timing of precipitation influences the susceptibility of diffuse knapweed to *L. minutus* attack. Such research would determine the resiliency of the biological control system to annual changes in weather patterns. I predict that knapweed plants subject to drought conditions during the early spring (when taproot resources are directed to vertical extension and apical bud growth) will be more affected by *L. minutus* than knapweed plants subject to drought conditions in the late spring or summer. The results of this research could further inform the model proposed earlier and could help to demonstrate how increased annual and intraseasonal variability in precipitation regimes could influence the efficacy of biological control.

### **Management implications**

Increased variability in weather patterns and other disturbance regimes will likely result in ecosystems more susceptible to the invasion of non-native species. Well-targeted biological control agents are likely to tolerate fluctuations in nutrient and water conditions of target host plants, and will be able to attack and cause damage to plants regardless of precipitation conditions. With increasingly variable weather patterns as predicted under global climate change, these results serve to underscore the importance of biological control as a tool for invasive species management.

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