DENSITY AND EFFICACY OF THE FLEA BEETLE *APHTHONA LACERTOSA* (ROSENHAUER), AN INTRODUCED BIOCONTROL AGENT FOR LEAFY SPURGE, IN ALBERTA

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B. Sc., University of Lethbridge, 1997

A Thesis
Submitted to the Council on Graduate Studies
of the University of Lethbridge
in Partial Fulfillment of the
Requirements for the degree of

MASTER OF SCIENCE

LETHBRIDGE, ALBERTA

MAY 2001

DEDICATION

"Your reason and your passion are the

rudder and the sails of your seafaring soul.

If either your sails or your rudder be

broken, you can but toss and drift, or else

be held at a standstill in mid-seas.

For reason, ruling alone, is a force confining;

and passion, unattended, is a flame

that burns to its own destruction."

(Kahlil Gibran, 1923)

To my mother -

thanks for mending the frays in my rudder and sails.

ABSTRACT

Biocontrol has been criticized because the target effects of biocontrol introductions have not been studied rigorously. The objectives of this thesis were 1) to assess quantitatively the efficacy of a classical biocontrol agent after its release and 2) to suggest factors that affect the density and distribution of the biocontrol agent. In 1997, *Aphthona lacertosa*, a root-feeding flea beetle that is native to Europe, was released for the biological control of leafy spurge in Alberta. The beetles had established at more than 75% of the release sites that were monitored in 1999. In 2000, the peak abundance of *A. lacertosa* across release sites ranged from low (<10 beetles m^{-2}) to high (>70 beetles m^{-2}). Sites with high beetle densities had a significantly greater local (ie. within 5m of release point) reduction of leafy spurge than sites with low beetle densities. The density and distribution of *A. lacertosa* are affected by cumulative degree-days (CDD) at the release site and plant morphology, respectively. Beetle population growth may be enhanced by releasing *A. lacertosa* at sites where there are more CDD. It is expected that high densities of *A. lacertosa* will effectively control leafy spurge populations.

ACKNOWLEDGEMENTS

I sincerely thank my supervisor, Rob Bourchier, whose support has made this project possible; working with Rob has been an invaluable learning experience. I would also like to thank my co-supervisor, Ralph Cartar for sharing his views of "the real world" - it's always fascinating to get a glimpse of what others see. My extreme gratitude is also extended to Rene Barendregt, Rose DeClerck-Floate, and Alec McClay for their support and valuable advice.

Financial support from Agriculture and Agri-Food Canada through the Federal Student Work Employment Program and Matching Investment Initiative was appreciated.

Thanks to those who released the beetles and collected field data in 1997; Cheryl Kasahoff, Kim Stromme and Jim Tansey, Alberta Agriculture Food and Rural Development under the supervision of Alec McClay, Alberta Research Council. I am eternally grateful to the County Fieldmen who helped me find the field sites. Thanks especially to the landowners whose enthusiasm, encouragement, hospitality, and humor kept me going.

I also thank those that helped me the most, both in the field and in the lab: Stephanie Erb, Kevin Floate, Peter Harris, Linda Van Heek, Ian Jonsen, Byron Lee, Jeff Rau, Alissa Reynolds, Stewart Rood, Monte Thomson, and Ray Wilson. Each person was instrumental in helping me to complete my project.

Finally, I thank my family for their interest and support. Thanks, dad, for your help and for building a cage to catch the little buggers! Uncle Ron, thanks for being the one family member to read everything I write - your support means a lot to me. Lisa and Melanie, thank-you for the many conversations and confirmation that you are going through similar experiences in completing your degrees. To Rocky, Kathi, Tyson, Kayla, and Wilco - thank-you for giving me more than my world of work.

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CHAPTER 1

Introduction and rationale

Weed biocontrol

Classical weed biological control is the introduction of non-native beneficial biocontrol agents (most often insects) to suppress foreign target weeds. Introduced weeds become a problem because they lack specialized natural enemies; classical biological control restores the ecological balance between the weed and herbivore populations. In Canada, classical weed biocontrol began in 1952 (Julien and Griffiths, 1998). Over 70 introduced insects have been released against 21 weeds and 2/3 of the biocontrol agents have successfully established (Julien and Griffiths, 1998; Harris 1991). Examples of weeds in Canada that show signs of decline from biocontrol agents include Dalmatian toadflax (*Linaria dalmatica* (L.) Mill), and hound's tongue (*Cynoglossum officinale* L.) (personal communication - Rose DeClerck-Floate, Agriculture and Agri-Food Canada, 2001).

Classical weed biological control releases are grand-scale field experiments and they provide a unique opportunity to study the population dynamics of herbivore-plant interactions. However, to date, there are very few long-term quantitative studies on the efficacy of weed biocontrol agents (McClay, 1995). Now that so many biocontrol agents have been released, established, and widely distributed, post-hoc monitoring studies are required to ensure the biocontrol agents are behaving as predicted and reducing target weed populations.

Leafy spurge biocontrol

Invasive weeds threaten Alberta's rangelands and native habitats. Leafy spurge is a noxious weed in Alberta (Weed Control Act, 1980) that covers more than 6 000 ha in central and southern parts of the province (McClay *et al.*, 1995). Leafy spurge reduces the utility of rangeland because the milky latex within leafy spurge plants is toxic and unpalatable to cattle (Kronberg *et al.*, 1993). Consequently, in pastures where there is more than 10% cover of leafy spurge, grazing is significantly reduced (Hein and Miller, 1992). Leafy spurge threatens native habitats by displacing native vegetation (Belcher and Wilson, 1989) and, that subsequently affects herbivores (Fownes and Bourchier, 2000; Trammel and Butler, 1995). Thus, leafy spurge causes economic losses on rangelands and environmental losses in biodiversity.

Leafy spurge will continue to spread without effective control. Reproduction of this aggressive perennial is by both vigorous lateral roots and seeds. Herbivores, wind, water, and contaminated crop seeds contribute to the spread of the weed (Noble, 1980; Selleck *et al.*, 1962; Bakke, 1936). Conventional control with herbicides may be effective (Lym and Messersmith, 1994), but application is often time consuming and expensive (Bangsund *et al.*, 1996; Watson, 1985). Further, herbicide application is difficult in some areas (eg. coulee hillsides) and herbicide application is not permitted in environmentally sensitive areas such as areas surrounding water bodies where leafy spurge is common. Leafy spurge is also highly tolerant of defoliation from herbicides and mowing (Alley and Messersmith 1985; Messersmith *et al.*, 1985). Additional control methods include competitive crops and sheep grazing which may be effective when combined with herbicides and maintained over several years (Landgraf *et al.*, 1984; Dercheid *et al.*, 1985).

An alternate, more cost-effective and long-term control solution for leafy spurge is biological control with natural arthropod enemies. In its native European habitat, leafy spurge is not considered a weed. In Europe, control of leafy spurge is probably the result of numerous specialist herbivores (Gassman and Schroeder, 1995). Eleven European insects, with a narrow host-feeding range, have been introduced into Alberta (Bourchier *et al.*, 2001). Success of the introduced agents has varied (Julien and Griffiths, 1998).

The most successful biocontrol agents of leafy spurge are those that feed and destroy the extensive root systems, preventing flowering of the mature plants and suckering of root buds. Such agents include the genus *Aphthona* which are root-feeding flea beetles. In Alberta, 5 European species of flea beetles have been released (Bourchier *et al.*, 2001). *Aphthona nigriscutis* is probably the most widespread flea beetle in the province; it is a promising biocontrol agent for leafy spurge at dry, exposed sites where it has reduced leafy spurge cover up to 80% (McClay *et al.*, 1995). The most recently released biocontrol agent for leafy spurge in Alberta is *A. lacertosa*. *Aphthona lacertosa* was chosen as an agent for release because it's better adapted to moist habitats (Gassmann *et al.*, 1996) than *A. nigriscutis*, and no previous leafy spurge biocontrol agent has been successful in moist habitats.

The effectiveness of *A. lacertosa* as a biocontrol agent for leafy spurge in Alberta has not been investigated. The main purpose of this thesis was to examine the efficacy of this flea beetle at release sites that were established throughout the province in 1997 by Alberta Agriculture Food and Rural Development. Sites were monitored in 1999 and 2000 for beetle and leafy spurge densities. Data on the current status and future prospects

of *A. lacertosa* as a biocontrol agent for leafy spurge in Alberta are presented in the following chapters:

Chapters 2 and 3 assess the current status of *A. lacertosa* release sites in Alberta. In Chapter 2, a degree-day model is used to assess the timing of the peak abundance of *A. lacertosa*. Data are presented on the proportion of sites that have beetle establishment and the relative abundance of the beetles across sites. Density data of the beetles in Chapter 2 are used in Chapter 3 to determine the efficacy of *A. lacertosa* as a biocontrol agent for leafy spurge.

Chapters 4 and 5 discuss factors that are important for consideration in future releases of *A. lacertosa*. Chapter 4 discusses the impacts of abiotic and biotic factors on flea beetle density at a site. Chapter 5 presents an experiment that was conducted to determine how host plant morphology affected beetle distributions and feeding preferences on leafy spurge shoots.

The final chapter, Chapter 6, summarizes the main conclusions of this thesis. The efficacy of *A. lacertosa* as a biocontrol agent for leafy spurge is discussed. In addition, recommendations for future releases and implications for leafy spurge and weed biocontrol are discussed.

The study system

Leafy spurge, *Euphorbia esula* L. (Euphorbiaceae) is an aggressive perennial that was first recorded in Alberta in 1933 (Haber, 1997). Leafy spurge reproduces both by seeds, which remain viable up to 8 years (Selleck *et al.*, 1962), and by vegetative root buds on rhizomes that extend laterally from a central tap root. The great genetic

variability in North American leafy spurge has resulted in taxonomic controversy as to whether leafy spurge is one species, or an aggregate of two or more European species (Geltman, 1998; Rowe *et al.*, 1997; Crompton *et al.*, 1990). Currently, North American leafy spurge is regarded as a single species, *Euphorbia esula*, and this taxonomy has been supported by morphological and gas chromatographic studies (Evans *et al.*, 1991; Crompton *et al.*, 1990).

Aphthona lacertosa (Chrysomelidae: Coleoptera), a black flea beetle from Eastern Europe, was first released in Canada in 1990 near Spruce Grove, Alberta for the biological control of leafy spurge (Bourchier *et al.*, 2001). The beetle has established in Manitoba, Saskatchewan, and Alberta (Julien and Griffiths, 1998). The lifecycle of *A. lacertosa* is similar to other univoltine *Aphthona* species. The beetle will adapt to both wet and dry habitats but the beetle prefers loamy soils (Gassmann *et al.*, 1996). The beetles are black and very small - about 2 mm. Their bodies are equally tapered in the front and rear (Harris, 2000) and the beetle jumps readily. The beetles over-winter as larvae in leafy spurge roots. Pupation and adult emergence occur in the late spring. Adult beetles mate and lay eggs just below the soil surface at the base of leafy spurge stems. *Aphthona* species lay eggs continuously in batches of a few hundred (Harris, 2000; Maw, 1981) for 2 months (Powell *et al.*, 1994). Eggs hatch and larvae migrate to the roots to feed.

Larvae of *A. lacertosa* cause the most damage to leafy spurge by feeding on the plant roots. Larvae feed on leafy spurge roots in the spring and fall, causing a reduction in water and nutrient absorption and this, in turn, leads to reduced plant height, flower development, and taproot strength (Rees *et al.*, 1996). *Aphthona lacertosa* adults also

cause damage to leafy spurge shoots by feeding on leafy spurge stems, leaves, and flowers. Beetle feeding causes a reduction in photosynthesis and flower production (Rees *et al.*, 1996).

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Chapter 2.

Post-hoc evaluation of *Aphthona lacertosa* establishment and density at leafy spurge biocontrol release sites

Abstract

The purpose of this chapter was to assess the establishment and densities of *Aphthona lacertosa* 3 years post release. In 1997, Alberta Agriculture and Rural Development released mixed populations of *Aphthona lacertosa* and *A. czwalinae* (Chrysomelidae: Coleoptera) at 94 locations in Alberta for the biological control of leafy spurge. By 1999 and 2000, beetle populations were composed primarily of *A. lacertosa*, with *A. czwalinae* contributing to less than 0.1% of the sampled populations. In 1999, 50 of the 1997 sites were monitored a single date and beetles had established at more than 75% of the sites. In 2000, 17 of the sites that were monitored in 1999 were monitored bi-monthly and beetles were found at every site. A degree-day model was used to assess the timing of the peak abundance of the beetles, which was expected at 1230 cumulative degree-days (CDD). Peak abundance of *A. lacertosa* in 2000 was low at 7 sites (< 10 beetles m^{-2}), moderate (10-70 beetles m^{-2}) at 4 sites, and high (>70 beetles m^{-2}) at 6 sites. Further analyses are required to assess the efficacy of *A. lacertosa* in reducing leafy spurge.

Introduction

Biocontrol practitioners have been successful at establishing biocontrol agents (Julien and Griffiths, 1998). The international weed biocontrol agent establishment rate

up to 1986 was 69% (Harris, 1991). Biocontrol agent establishment and density data are required to assess the efficacy of the agent.

A degree-day model may serve as a tool to assist in the collection of density data for biocontrol agents. If the release agent is an insect, temperature is important in predicting changes in its population. Insects are poikilothermic and as such, development and growth are related to temperature (Higley *et al.* 1986). Degree-day models use temperature data and the insect's physiology to predict emergence (Beers *et al.* 1993). There are very few examples in the biocontrol literature that use a degree-day model for predicting insect phenology (but see McClay and Hughes, 1995). In contrast, degree-day models are common to integrated pest management approaches for the prediction of pest outbreaks (Ro *et al.*, 1998; Ferro, 1994).

In this chapter, a degree-day model is used to assess the timing of the peak abundance of *Aphthona lacertosa*, a biocontrol agent for leafy spurge. The goals of this chapter were twofold: 1) to determine the proportion of sites where beetles successfully established in Alberta and 2) to determine the relative abundance of the beetles across the release sites.

Methods

Collection of field data

In 1997, Alberta Agriculture Food and Rural Development released 94 mixed populations of *A. lacertosa* and *A. czwalinae* (Chrysomelidae: Coleoptera) in Alberta, Canada for the biological control of leafy spurge. The relative proportion of each species of beetle in the releases was unknown because the beetles are morphologically similar

(LeSage, 1996). The original source of the beetles was Valley City, North Dakota, USA where beetles were collected on June 24 and 25, 1997. Beetles were released over a period of 12 days between June 27 and July 7, 1997 on a variety of prairie landscapes including coulee hillsides, alluvial floodplains, rangelands, croplands, and railway and road ditches. Release sites were primarily on privately owned land throughout southern and central Alberta. The criteria for selecting release sites were that the habitat should be shaded and preferentially mesic with loamy soils. Most releases (n=37) consisted of 1000 beetles but some releases (n=13) consisted of 2000 beetles. In 1999, there were no significant differences between mean beetle densities at sites where 1000 or 2000 beetles were released (Mann-Whitney U=185, P=0.558).

The appropriate timing for sampling populations of *A. lacertosa* was unknown because, prior to this study, no specific information was available on the phenology of *A. lacertosa* in North America. The beetles were reported to emerge at the beginning of June and persist for about 2 months (Harris, 2000). Sample dates affect the number of beetles that are present at a site. Sampling sites too early or too late in the season would result in an underestimate of the number of beetles at a site and these data would not be useful to compare densities between sites without the knowledge of site-specific beetle phenology. Two approaches were taken in 2 years to quantify the beetle densities across sites.

The first approach, in 1999, involved taking a single sample of beetle densities at as many sites as possible (n=50). The advantage of this sampling method was that many sites could be sampled over a large geographical area and it was possible to examine sites with a wide range of climate and habitat. Site locations were recorded using a handheld

GPS (Garmin 12XL). These extended from Coleman in the west (49.6°N, 114.5°W) to Medicine Hat in the east (50.2 °N, 111.5 °W) and from Cardston in the south (49.2 °N, 113.3 °W) to Brosseau in the north (53.5°N, 111.3°W) (Fig 2.1a). Sites were monitored for *A. lacertosa* densities once in 1999 between June 29 and July 29. Southern sites, were monitored first because they were expected to have earlier beetle emergence than northern sites.

The second approach, in 2000, involved taking multiple samples of beetle densities on as many days as possible, but at fewer sites (n=17) than in 1999 (Fig. 2.1b). The goal of the intensive sampling was to sample sites when *A. lacertosa* individuals were at similar stages of development among sites to remove beetle phenology as a factor in explaining density variation across sites. Sites were chosen to represent the broad geographical distribution of the sites across Alberta and to represent the diversity of beetle densities in 1999. Start date for 2000 sampling was based on degree-day modeling; beetle densities were sampled bi-monthly, using the same methods as in 1999, from June 5 to September 5, 2000 (Table 2.1).

At all sites, beetle densities were assessed in a fixed area with a similar sampling effort. A garbage can with the bottom cut off (diameter 41.5 cm, area 0.127 m^2) was placed over the plants at 5 and 10 m along the transect line in each of the cardinal directions from the release point. The area inside the garbage can was vacuumed using a modified leaf blower (STIHL BG75, high idle flow rate=0.25 cubic meters/minute) for 45 seconds. Vacuuming was vigorous and efforts were made to vacuum the leafy spurge shoots, the sides of the can, and the ground to include beetles that had jumped off the plants or fallen to the ground. Vacuum samples of beetles were collected in a fine mesh

stocking and placed on ice. At the lab, samples were frozen and then sorted to quantify and dissect *A. lacertosa*.

Establishment of *A. lacertosa* in Alberta

Beetles collected in 1999 and 2000 were examined to determine their species. Species were distinguished by examining the color of the 6th leg segment from the bottom of the leg under a dissecting microscope. *A. czwalinae* have dark-colored hind tibia while *A. lacertosa* have yellowish tibia (sic., LeSage, 1996). The proportions of each beetle species for all beetle release sites in each year were calculated.

Mean beetle densities of *A. lacertosa* were plotted using the site coordinates. Mean beetle densities in 1999 and 2000 were calculated using the number of beetles vacuumed at 5 m in each direction for each sample date at each site. Density data were divided into categorical data because of the difficulties of obtaining representative beetle densities at the sites. Sites were divided into low (<10 beetles/m^2), moderate (10-70 beetles/m^2) and high (>70 beetles/m^2) *A. lacertosa* density sites (Table 2.1). The groupings best described the peaks in the frequency distribution of beetle densities in 1999 and 2000 (Fig. 2.2). Sites with low, moderate, and high beetle densities were visually distinct in the field. At low-density sites in 1999 and 2000, there was no visible damage to leafy spurge shoots and no beetles were visible. Moderate density sites had visibly damaged leafy spurge shoots and the beetles were easy to find. High-density sites were "outbreak" sites where the beetles were so numerous that leafy spurge shoots were completely covered with beetles and many leafy spurge shoots were totally defoliated by the beetles.

Changes in beetle densities at the 17 release sites monitored in both 1999 and 2000 were compared to determine how closely the single samples in 1999 estimated the peak beetle densities in 2000. The purpose of this comparison was to determine whether the 1999 data could be used to compare beetle densities across the release sites in Alberta. It was predicted that in 1999, release sites were sampled at various stages of beetle phenology and the beetle densities probably did not describe the relative beetle abundance at a site.

The degree-day model

In the winter of 1999, an unpublished degree-day model was obtained to predict when peak abundances of the beetles should occur at sites in Alberta. The model was developed by Rich Hansen (United States Department of Agriculture, Animal and Plant Health Inspection Service, Montana) to predict peak abundances of *A. lacertosa* at field sites in Montana. The degree-day model was developed, tested, and validated as a good model for Montana populations of *A. lacertosa* (personal communication - Rich Hansen, United States Department of Agriculture, 2001) that came from the same source as the Alberta beetles (ie. Valley City, North Dakota, USA). The model assumed that *A. lacertosa* development had a positive, linear relationship with temperature, which was deemed a reasonable assumption for *Aphthona* species in North America. The model calculated average cumulative degree days (CDD) using the half sine wave method (Allen, 1976) with January 1 as day 1 and a threshold temperature of 0 °C. The sine-wave method of calculating degree-days assumes that the rise and fall of daily temperatures approximates a sine wave pattern. Using daily maximum and minimum

temperatures, CDD are mathematically calculated (see multiple equation calculations in Allen, 1976). Based on field populations in Montana, peak densities of *A. lacertosa* were expected at 1230 CDD.

All temperature data required for CDD calculations were obtained from Environment Canada weather stations because no site-specific temperature data were available. *Aphthona lacertosa* release sites were matched with the nearest weather station. Daily maximum and minimum temperatures were obtained from Environment Canada at 11 stations in Alberta and one station in British Columbia (Table 2.2). At release sites where no station was within the vicinity (i.e. within 200 km), temperature data were interpolated between the 2 nearest weather stations (Table 2.2). Interpolated data were calculated as arithmetic mean temperatures of the 2 stations.

Application of the degree-day model

The degree-day model was used as a tool for several purposes. Beetle density data from 1999 were fitted to the degree-day model to determine how close the sampling dates were to the expected peak date of beetle abundance. The purpose of fitting this data to the model was to predict whether beetle densities in 1999 might have been underestimated as a result of sampling too early or too late in the season. *Aphthona lacertosa* densities were plotted against 1999 CDD. The number of days that each sampling date was away from the predicted peak sampling date was calculated and plotted.

The degree-day model was used to design the time frame for sampling Alberta release sites for beetle densities in 2000. Mean CDD and Julian dates from 1980-1990

for the weather stations were plotted, and the dates that corresponded to the estimated peak density value of 1230 CDD were determined. It was expected that averaging 10 years of weather data would take into account the year-to-year variation in the weather and provide a good approximation of the dates when *A. lacertosa* populations should be sampled in 2000.

Beetle densities from 2000 were fit to the degree-day model to confirm that the model accurately predicted the date of peak beetle abundance at release sites. The average CDD of peak beetle peak abundance at release sites was compared to the expected 1230 CDD value using a T-test. Twelve release sites were used in this analysis because peak beetle abundance was not measured at 5 release sites (sites 111, 158, 153, 151, 155).

Results

Establishment of *A. lacertosa* in Alberta

Beetle populations in Alberta were primarily composed of *A. lacertosa*. *A. czwalinae* accounted for less than 0.5% (n=955) and 0.4% (n=833) of all the beetles collected from the release sites in 1999 and 2000, respectively.

In 1999, sites with the highest densities of *A. lacertosa* were south of Calgary (Fig. 2.1a). Beetle densities were low (<10 beetle/m^2) at 86% (n=43 sites) of the sites. Beetles were not found at 14 of the low-density sites on the single date that the site was monitored. There were 4 moderate density sites: two in Magrath and one each in Taber and Ft. Macleod. High beetle density sites were at Lethbridge, Millarville, and Pincher

Creek. At the 3 of these sites, the mean density of *A. lacertosa* was about 84.9 m^{-2} (± 0.20 SE).

In 2000, beetles were found on at least one sample date at every site that was monitored. Peak beetle densities ranged from 2-1500/ m^2 across sites (Table 2.1 – see footnote 'c'). Low beetle densities were found at 40% of the sites that were monitored in 2000. All high beetle density sites were south of Calgary. The site with the highest density of beetles was Millarville. Other high densities sites were Bow Island, Taber, Lethbridge, Ft. Macleod, and Cardston. Peak beetle densities at these sites ranged from 80 to 250 beetles/ m^2.

Beetle density estimates were compared at sites that were monitored in both years to determine how reliable single density samples were. In 2000, based on peak densities, more sites had moderate to high beetle densities than in 1999 (Table 2.1). Eight of the 17 sites monitored in 2000 increased in density classification. Four sites went from low density classification in 1999 to moderate density in 2000, 3 sites went from moderate density to high density, and one site went from low to high density classification (Table 2.1). None of the 17 sites decreased in density classification from 1999 to 2000.

Application of the degree-day model

The degree-day model was used to assess how close 1999 sampling dates were to the expected peak abundance date. CDD of the sample dates ranged from approximately 900 to 1400 CDD (Fig. 2.3a). The three sites with the highest beetle densities had between 1000 and 1150 CDD and were sampled approximately 5 to 15 days ahead of the

predicted peak density (Fig. 2.3b). More than 50% of the moderate/high density sites were monitored within 10 days of the estimated peak CDD date. Sites that were sampled more than 2 weeks ahead of the predicted peak were south and west of Lethbridge.

The degree-day model was used to decide when to begin sampling in 2000. The model suggested that *A. lacertosa* should reach peak abundance in Alberta between June 28 and July 21, depending on the site location (Fig. 2.4). However, *A. lacertosa* emerged earlier than expected in 2000. Sampling began June 5, but the peak abundance of beetle populations may have occurred on or before the first sampling date at sites near Bow Island, Taber and Lethbridge because all beetle densities after the first sample date declined (Table 2.1).

The degree-day model was also used to confirm that beetles had similar phenology across sites. In 2000, beetle populations had an average peak at 1178 CDD (± 73.4 SE) (Fig. 2.5). The mean CDD that beetles were at peak abundance at the release sites was not significantly different from the expected 1230 CDD (T-test: n=12, t=0.24, P>0.5).

Discussion

Two approaches were taken in 2 years to assess beetle establishment and densities at sites across Alberta. In 1999, single sample dates were too early at many sites and as shown by the comparison of 1999 and 2000 densities, many of the beetle densities at sites were probably underestimated in 1999. Although beetle density increases were expected between 1999 and 2000, it was uncertain how much of the increase would be the result of population growth. Thus, the 1999 density data were not useful for comparing *A.*

lacertosa densities across sites. However, most of the single sample dates in 1999 were useful to determine whether *A. lacertosa* had established at release sites throughout the province, with the exception of the sites that were sampled early. It was concluded that *A. lacertosa* successfully established at more than 75% of the release sites throughout the province.

The degree-day model predicted the dates that peak beetle abundances occurred at sites across Alberta. In 2000, densities of *A. lacertosa* peaked between 800 and 1300 CDD, peaking on average 80 CDD or about 5 days earlier than predicted. Drought conditions in southern Alberta may have contributed to the earlier than expected beetle emergence in the south. Additionally, differences in the predictions of CDD requirements for peak beetle abundance between the predicted and expected values as well as between the populations in 2000 may have occurred for several reasons. First, although the model was designed for *A. lacertosa*, some *A. czwalinae* were probably included in the 1230 CDD prediction (personal communication - Rich Hansen, United States Department of Agriculture, 2001). Thus, differences in the proportion of the 2 species in populations may account for different peak estimates. Second, the frequency of sampling dates differed between estimates. The model was designed with weekly or bi-weekly sampling intervals (Rich Hansen, personal communication) while sampling in 2000 was bimonthly. Different sampling intervals probably affected the accuracy of when the estimated peak abundances occurred. Third, differences in CDD requirements can probably be attributed to the estimation of the beetles' thermal environment. Deviation in predictions may have been reduced if site-specific temperature data were used. Further, temperature predictions of thermal environments does not account for

different amounts of rainfall or soil types, both of which vary across the province (Alberta Agriculture, 1995; Dzikowski and Heywood, 1990) and affect the beetles' thermal environment. Even with the number of sources for variation in the CDD estimates, the CDD estimates were similar to the predicted 1230 value (Fig. 2.5) and thus, the model provided an operationally useful estimate of the dates when high beetle abundances would occur.

In this chapter, it was shown that *A. lacertosa* successfully established at the majority of the release sites in Alberta. The beetle densities at peak abundance in 2000 provided a useful measurement of comparing beetle densities across release sites in Alberta; the beetle densities were moderate or high at 60% of the sites. Despite these impressive statistics, based on these data alone, it is not possible to conclude that *A. lacertosa* is an effective biocontrol agent.

Conventional wisdom in weed biocontrol suggested that as biocontrol agent densities increased, more damage would accumulate on the target weed, and biocontrol would be achieved. However, this was not always the case; only 15-20% of weed biocontrol agents successfully reduced their target population (Myers *et al.*, 1988). Biocontrol agents will be unsuccessful at reducing their host weed unless they damage a part of the weed that regulates the weed population (Crawley, 1989). Two examples of biocontrol agents that successfully established and damaged, but did not reduce their host weed populations are the spurge hawk (*Hyles euphorbiae* (L.)) and cinnabar moths (*Tyria jacobaeae* (L.)). The cinnabar moth was released for the biocontrol of tansy ragwort, and even through the larvae defoliated plants, the plants compensated without a long-term reduction in plant density (Myers 1980). Similarly, the spurge hawk moth is widespread

in Alberta on leafy spurge (personal observation) and causes noticeable feeding damage, but leafy spurge infestation is not reduced by defoliation (Rees *et al.*, 1996).

In conclusion, further assessment is required to determine whether *A. lacertosa* has caused a reduction in leafy spurge. Combining the beetle density data from this chapter with density data for leafy spurge will enable the assessment of the efficacy of *A. lacertosa* and this assessment is provided in Chapter 3.

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Table 2.1 Peak densities (m^{-2}) of *Aphthona lacertosa* sampled at biocontrol release sites in Alberta in 1999 and 2000. Sample date(s) that had the highest average density of beetles (n=4 samples per site at 5m north, south, east, and west) is/are underlined.

Release site ID	Near Town	Location (N, W)	2000 Julian sample dates							2000 Peak density	Density class[1] 2000	Density class[1] 1999
153	Bow Island	50.0, 111.0	<u>159</u>	172	189	201	215	--	--	81	H	L
151	Bow Island	49.9, 111.5	<u>162</u>	172	189	201	215	--	--	41	M	L
155	Taber	49.8, 112.2	<u>159</u>	172	189	201	215	--	--	30	M	L
158	Taber	49.8, 112.4	<u>159</u>	172	189	201	215	--	--	224	H	M
118	Lethbridge	50.1, 112.9	157	<u>174</u>	187	200	213	--	--	2	L	L
013	Lethbridge	49.7, 112.9	157	<u>171</u>	187	200	213	--	--	22	H [a]	H
111	Ft. Macleod	49.7, 113.3	--	<u>173</u>	188	202	214	--	--	250	H	M
114	Ft. Macleod	49.7, 113.2	161	<u>173</u>	<u>190</u>	202	214	--	--	2	L	L
163	Magrath	49.4, 113.0	157	174	<u>187</u>	200	213	228	244	39	H [b]	M
123	Cardston	49.2, 113.3	157	171	187	<u>200</u>	213	228	244	59	M	L
124	Pincher Creek	49.7, 114.1	161	173	<u>188</u>	<u>202</u>	214	--	--	2	L	L
128	Coleman	49.6, 114.5	--	175	190	<u>202</u>	214	--	--	4	L	L
131	Millarville	50.8, 114.3	165	179	<u>208</u>	222	--	235	250	141	H [c]	H
021	Red Deer	52.3, 113.9	165	178	<u>208</u>	<u>221</u>	--	234	249	11	L	L
171	Three Hills	51.6, 113.2	167	178	208	<u>221</u>	--	234	249	2	L	L
020	Mirror	52.5, 113.1	166	178	207	<u>221</u>	--	234	249	20	L	L
018	Camrose	52.9, 112.8	166	178	<u>207</u>	221	--	234	249	70	M	L

[1]Sites were classified by peak densities as high (H) : > 70 beetles/ m^2, moderate (M) : 10-70 beetles/ m^2, and low (L) : < 10 beetles / m^2.

[a] Beetle densities in 1999 were high. With little leafy spurge in 2000, beetles have moved away from release area.

[b] Beetles moved from release point about 13 m NE, moving up a coulee hill.

[c] Beetles moved outside of sampling area because there is no leafy spurge. On July 26 (julien date 208), a single sample outside of the halo, where spurge was still present had 1500 beetles/m^2.

Table 2.2 Location of Environment Canada climate stations used to calculate cumulative degree days (CDD) at 1997 *Aphthona lacertosa* release sites in Alberta. Maximum and minimum daily temperatures were used to calculate CDD for the years indicated. Climate stations were matched with the town nearest the release sites, and in some cases, the average of the two stations was used to approximate the latitude of the site.

Climate station	Location (N, W)	Years	Near Towns	Release site ID
Calgary	50.1, 114.0	1980-1990,1999,2000	Millarville	131
Camrose	53.0, 112.8	1980-1990,1999,2000	Camrose	18
Cardston	49.2, 113.3	1980-1990,1999,2000	Cardston, Magrath	119,122,123,160,163
Claresholm	50.0, 113.7	1980-1990,1999,2000		--
Cardston + Claresholm		1980-1990,1999,2000	Ft. Macleod	110-115
Edmonton	53.3, 113.6	1980-1990,1999	Sherwood Park, Brosseau	301,305
Lethbridge	49.7, 112.8	1980-1990,1999,2000	Lethbridge	11,13-16,116-118
Lloydminster	53.3, 110.0	1983-1990,1999		--
Lloydminster + Red Deer		1983-1990,1999	Hardisty	35,36,42
Medicine Hat	50.0, 110.7	1980-1990,1999,2000	Seven Persons, Medicine Hat	104,106-108
Olds	51.8, 114.1	1980-1990,1999,2000	Three Hills	171,172
Pincher Creek	49.5, 114.0	1980-1990,1999,2000	Pincher Creek, Lundbreck	124-127,130
Red Deer	52.2, 113.9	1980-1990,1999,2000	Red Deer	21
Red Deer + Camrose		1980-1990,1999,2000	Mirror, Ponoka	20,24
Taber	49.8, 112.1	1980-1990,1999,2000	Taber	154-159
Taber + Medicine Hat		1980-1990,1999,2000	Bow Island	151-153
Crowsnest Pass		1999, 2000	Coleman	128

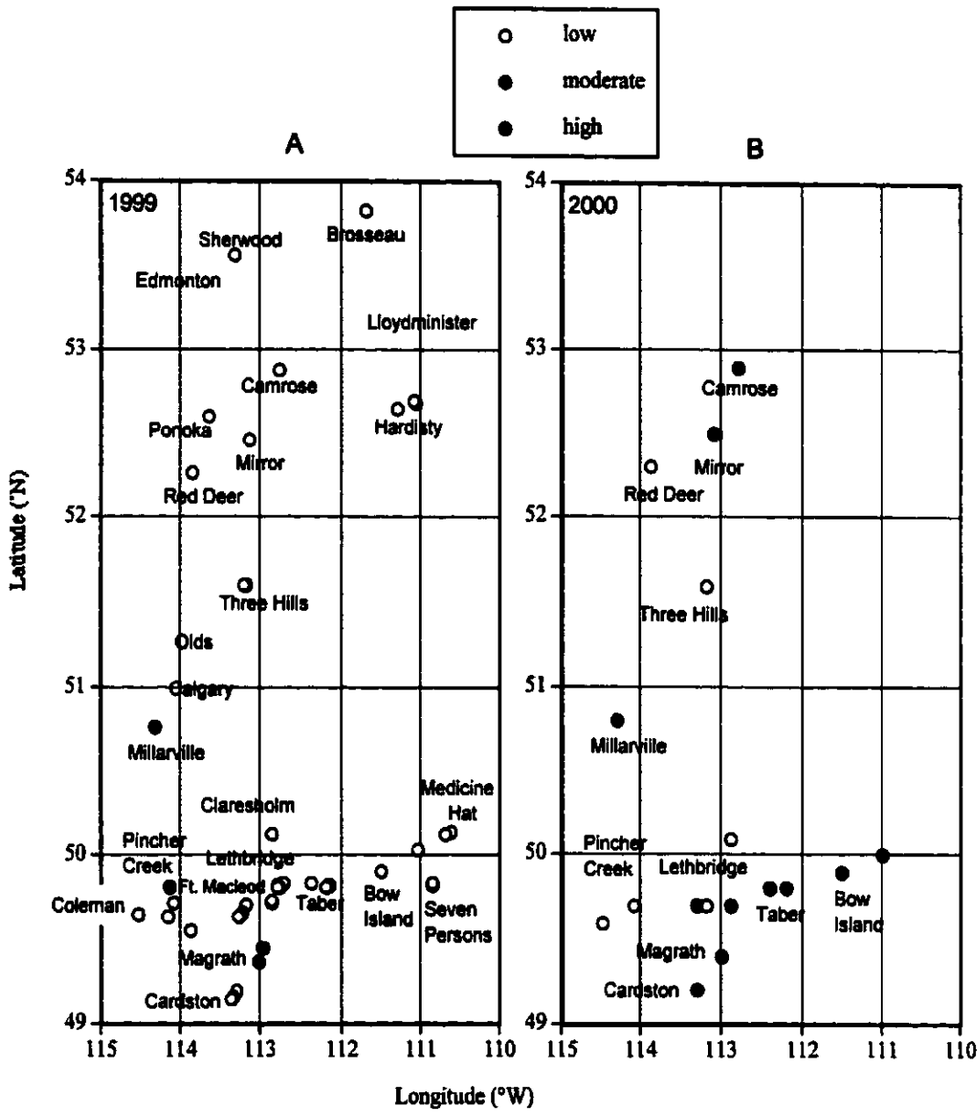

Figure 2.1A: Location of 1997 *Aphthona lacertosa* releases in Alberta monitored in 1999 (n=50) with mean beetle densities from a single sample date.
2.1B: Location of the same sites monitored in 2000 (n=17) with mean beetle peak densities from bi-monthly sampling.
The approximate location of cities and towns are indicated and beetle densities are indicated in Table 2.1

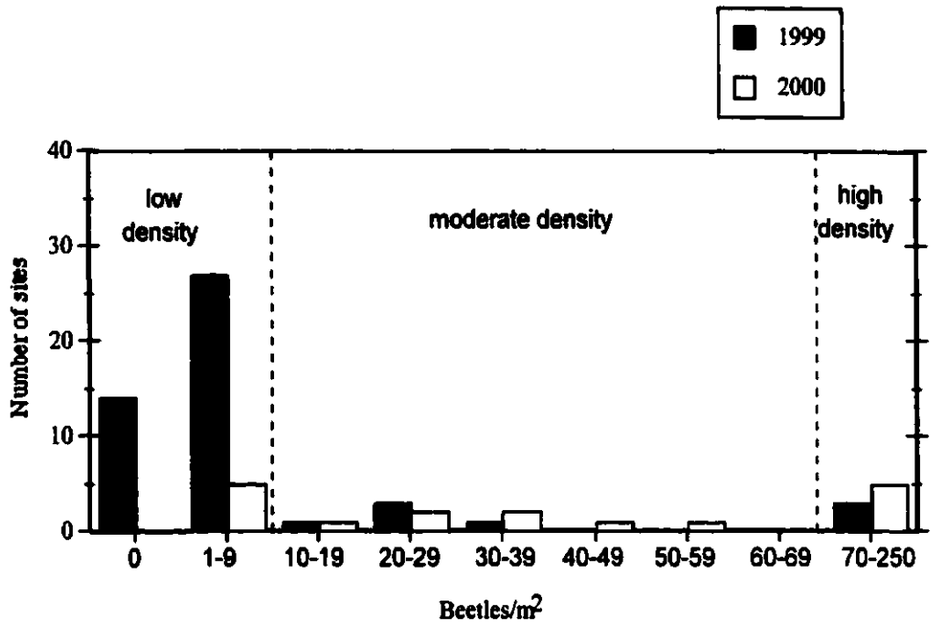

Figure 2.2 Mean *Aphthona lacertosa* densities at biocontrol release sites in Alberta in 1999 (n=50) and 2000 (n=17). For data analyses, sites were divided in to those with low (<10 beetles/m^2), moderate (10-70 beetles/m2) and high (>70 beetles/m^2) beetle densities.

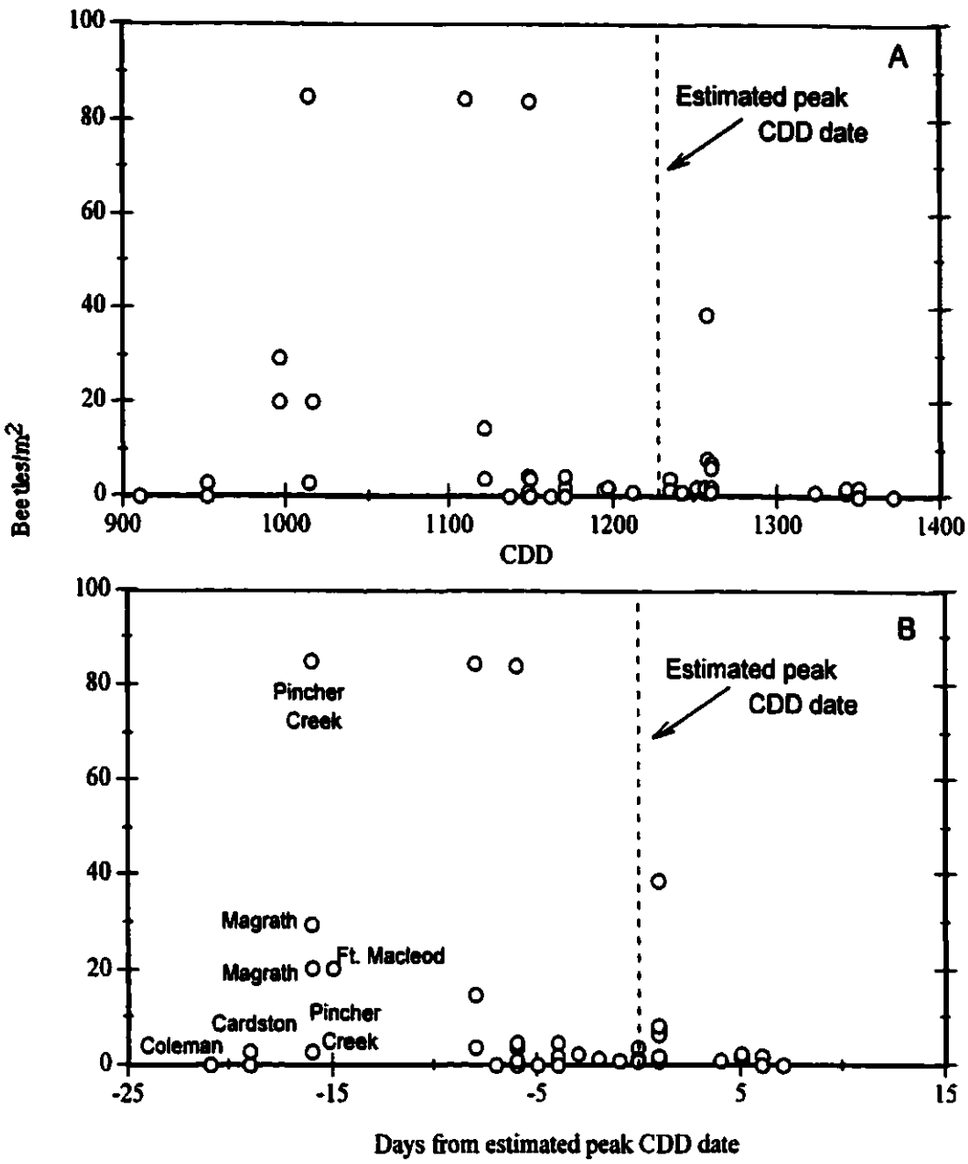

Figure 2.3A: Mean 1999 *Aphthona lacertosa* densities at Alberta sites (n=50) and the cumulative degree-days (CDD) on the date of monitoring. Peak beetle densities should occur at approximately 1230 CDD.
2.3B: The number of days that sampling differed from the estimated peak CDD date for each of the sites is indicated with negative days indicating early sampling and positive days indicating late sampling. Locations of sites that were monitored more than 10 days off the estimated peak CDD are listed.

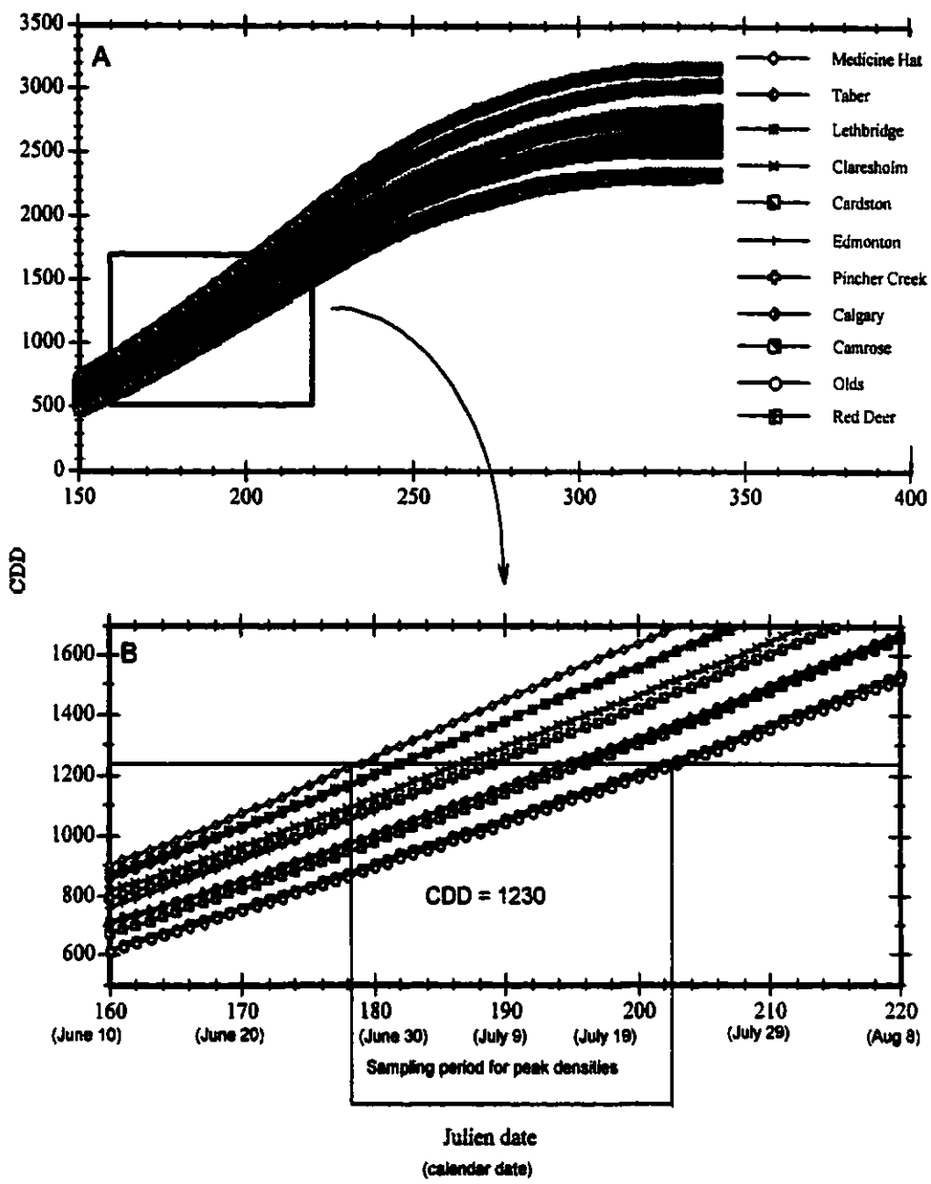

Figure 2.4A: Mean (1980-1990) cumulative degree-days (CDD) for the closest Alberta weather station to *Aphthona lacertosa* release sites.
2.4B: The estimated peak density of the beetles is 1230 CDD; based on this estimate, peak densities at the release sites should occur between June 28 to July 21.

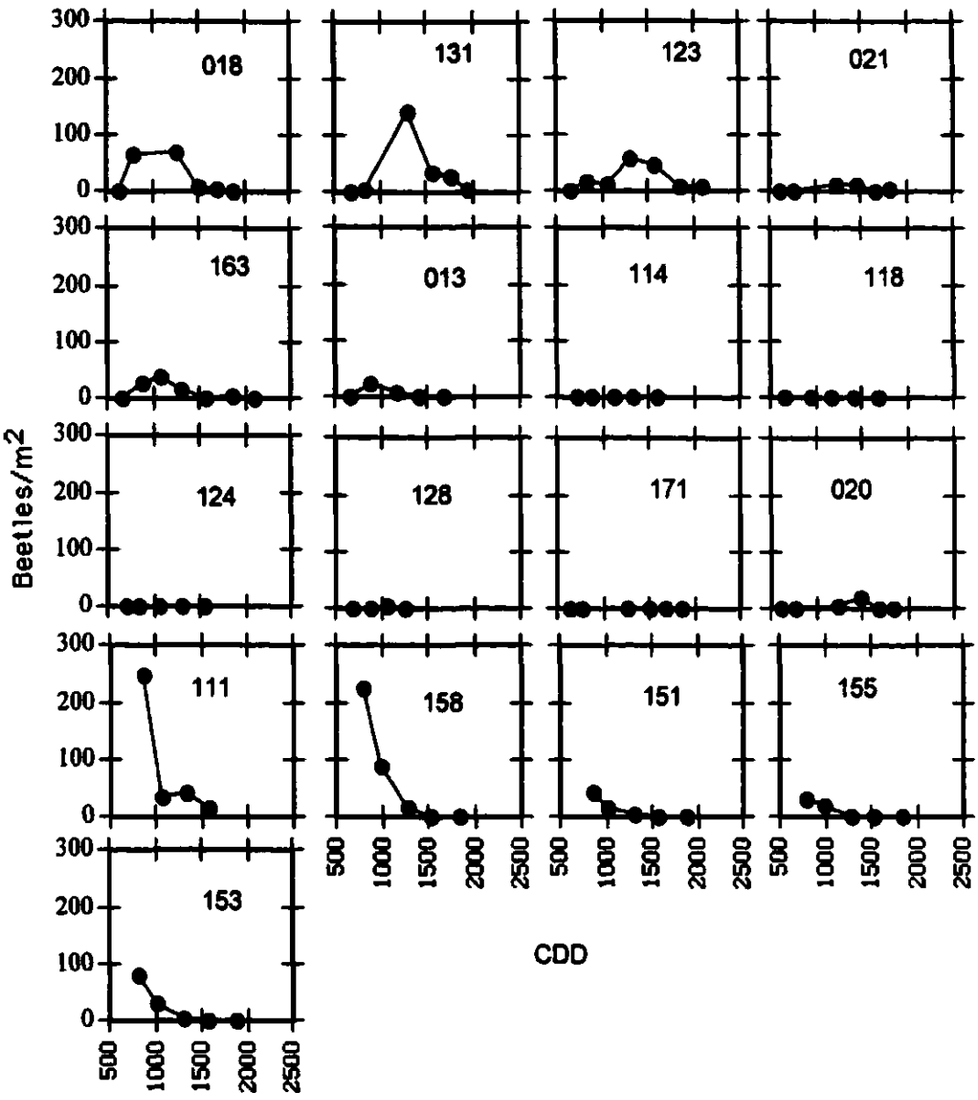

Figure 2.5 Change in *Aphthona lacertosa* populations at 17 leafy spurge biocontrol release sites in Alberta that were monitored in 2000, bi-monthly. Cumulative degree-days (CDD) for each site were calculated using the nearest climate station (see Table 2.2). Points represent actual estimates and lines connect points from the same site.

Chapter 3.

Post-hoc evaluation of the efficacy of *Aphthona lacertosa* at leafy spurge biocontrol release sites

Abstract

The efficacy of *Aphthona lacertosa*, a biological control agent for leafy spurge, was assessed 3 years post release. Alberta Agriculture Food and Rural Development collected weed density data at 17 sites in Alberta in 1997, immediately prior to the release of the beetles. Weed density data was collected again in 2000. ANOVA's were used to assess the changes in leafy spurge percent cover, stem density, and canopy height at sites with low (7 sites with < 10 beetles m^{-2}), moderate (4 sites with 10-70 beetles m^{-2}), and high (6 sites with >70 beetles m^{-2}) beetle densities. Sites with high beetle densities had significantly greater reductions of leafy spurge within 5 m of the release point than sites with low beetle densities ($P<0.017$). At high beetle density sites, leafy spurge cover decreased by 46.8% ($\pm$ 9.7 SE), stem density decreased by 97 stems per m^{-2} ($\pm$18 SE), and leafy spurge height decreased by 57.3 cm ($\pm$7.6 SE). Damage caused by the beetles at high-density sites was often visible as a dead, "halo" shaped patch of leafy spurge shoots around the release point. Locations of the reductions in leafy spurge were not predictable; new leafy spurge shoots were quick to grow and the beetles did not necessarily disperse randomly from the release point. It is predicted that continued impact by *A. lacertosa* will result in leafy spurge biological control at high and moderate beetle density sites.

Introduction

Biological control has often been criticized because the outcomes of many biocontrol introductions have not been rigorously studied (Pearson *et al.*, 2000; Callaway *et al.*, 1999; McEvoy and Coombs, 1999). In many cases, the lack of rigorous study relates to poor experimental design prior to release of the biocontrol agent (McClay, 1995) or to the differing objectives between applied practitioners and researchers. Practitioners in biocontrol focus on spreading the biocontrol agent and this is often counterproductive to the experimental approaches favored by researchers (Harris, 1991).

A common problem when trying to analyze the impact of a biocontrol agent is the lack of control sites (McClay, 1995). It is often impossible to establish control sites post-release because the biocontrol agents disperse. In such situations, it is a challenge for biocontrol practitioners to provide evidence that a biocontrol agent has caused a change in the weed density, rather than the change being caused by other factors like weather, soil conditions, and/or competitors.

In the literature, the efficacy of herbivores in reducing plant populations is most often shown in two ways. First, data are often presented that show that as insect herbivore density increases, weed density decreases (Louda and Potvin, 1995; Prins *et al.*, 1989; and Whittaker, 1982). Another way to show biocontrol agent efficacy is to present weed density data or photographs from "before and after" the release (McClay, 1995). In this study, both of these approaches are employed to show the relationship between a biocontrol agent, *Aphthona lacertosa*, and its target weed, leafy spurge.

Other *Aphthona* species have previously been recognized as useful biocontrol agents for leafy spurge (Gassman and Schroeder, 1995). Suppression of leafy spurge populations at some sites in Alberta has been attributed to *A. nigriscutis* (McClay *et al.*, 1995). A single study has examined the impact of *A. lacertosa* on leafy spurge in North Dakota 4 years post release (Kirby *et al.*, 2000). *Aphthona lacertosa,* in combination with *A. czwalinae,* caused a significant reduction in leafy spurge cover, density, yield, root density, root weight and the number of root buds when compared to beetle-free control sites (Kirby *et al.*, 2000).

Releases of *A. lacertosa* in Alberta commenced in 1997 in an attempt to mimic the successes achieved in North Dakota. This chapter reports the changes in leafy spurge populations caused by *A. lacertosa* at release sites in Alberta 3 years post release. It was expected that the beetles had caused a reduction in the leafy spurge and these reductions would be greatest closest to the point where the beetles were released.

Methods

Collection of field data

Consideration of the biology of A. *lacertosa* and leafy spurge was required prior to the collection of density data. When showing a relationship between biocontrol agent density and weed damage, biocontrol agent density should be measured when the agent is in its damaging life form. In the case of *A. lacertosa*, the damaging life form is the larva feeding on the roots of leafy spurge. However, it was not practical to assess larval densities due to the difficulties of excavating leafy spurge roots that may extend more

than 7 m deep (Whitson *et al.*, 1996) in hard, compact soils. Thus, *Aphthona* beetle densities were used as a surrogate of larvae densities in this study as was done in previous studies (Kirby *et al.*, 2000; McClay *et al.*, 1995). The selection of the dates to sample weed densities was problematic because there is a time delay between when the root damage happens and when the damage is visible. *Aphthona lacertosa* larvae feed on leafy spurge roots in the spring and fall but leafy spurge shoots do not immediately die and thus, shoots that have damaged roots may look healthy. It was decided that the relationship between biocontrol agent density and weed declines was best described by measuring leafy spurge density in the mid-summer because the root damage caused by the larvae in the spring and fall would be evident in mid-summer.

Leafy spurge and beetle density data were collected in 2000 to determine the efficacy of *A. lacertosa* at 1997 Alberta release sites. Beetle densities were measured as previously described (Chapter 2). In 2000, leafy spurge densities were measured at 17 sites during the month of July. Sampling methods were consistent across sites and were based on the techniques used by Alberta Agriculture Food and Rural Development to measure leafy spurge density in 1997, prior to the release of the flea beetles at sites (personal communication - Jim Tansey, Alberta Agriculture Food and Rural Development, 1999). All sampling occurred in a clockwise direction either centered on, or right of the transect line in each of the 4 cardinal directions from the release point. Leafy spurge percent cover and the number of vegetative and flowering shoots were measured using quadrats (50 x 20 cm) that were placed along the transect line at 1, 3, 5, and 10 m from the release point. The average height of the leafy spurge canopy within 5

m of the release point was recorded and photographs of the area around the release point were taken at each site.

In 1999, beetle impact was visible by a patch of dead leafy spurge that usually extended from the point of release in a characteristic circular-shaped "halo". Halos were not always around the point of release but often were on the uphill side of the release. Halo presence and absence was recorded at all sites in 1999 and 2000.

Statistical analyses

Impact of the beetles on leafy spurge in 1999 was examined by analyzing halo data. The relationship between 1999 beetle densities and the presence or absence of a halo was assessed using logistic regression.

The impact of the beetles on leafy spurge was assessed by comparing height, percent cover, and stem count data from 1997 and 2000 at 17 sites. The best measure of impact on leafy spurge is the change in stem density, since height is seasonally variable and percent cover estimates can vary greatly between observers. However, data on leafy spurge stem counts were not available for all sites in both 1997 and 2000. Missing data in 2000 can be attributed to site disturbance or drought conditions that caused leafy spurge to dry out and crumble before measurements could be taken. As a solution, all three leafy spurge attributes were analyzed with the expectation that there should be similar trends in the decreases of leafy spurge height, cover and stem counts if the beetles were having an impact.

Changes in leafy spurge canopy height from 1997, immediately prior to the release of the beetles, to 2000 were compared across sites with varying beetle densities.

An ANOVA was conducted with the response variable being the change in the canopy height of leafy spurge tested against the fixed factor of beetle density in 2000 (low, moderate, and high). Differences between treatment means were assessed using Tukey's HSD test (α=0.05).

Changes in leafy spurge percent cover and stem count from 1997 to 2000 were compared across sites with varying beetle densities and at 3 distances from the release point. The mean change per site of the percent cover and the number of stems (flowering and vegetative combined) of leafy spurge were calculated by pooling across the directions north, south, east, and west. Two split-plot ANOVA's (Zar, 1999) were conducted with site as a random factor and nested within beetle density. The response variables were either the change in mean percent cover or the change in mean stem number tested against the fixed factors of beetle density in 2000 (low: <10 beetles m^{-2}, moderate: 10-70 beetles m^{-2}, and high: >70 beetles m^{-2}) and distance (1, 3, 5 m from the release point). Site is used as part of the mean squares error term to calculate the F-ratios for distance and the interaction of density*distance. Differences between treatment means were assessed using Tukey's HSD test (α=0.05).

Results

Halos were present at 12 of the 50 sites monitored in 1999. It was unusual to find beetles inside the halo because there were few living leafy spurge shoots. Instead, beetles were found most often on the advancing edge of the halo. The presence of a halo was positively related to beetle density (logistic regression: n=50, χ^2=74.96 , P<0.000). In 1999, at sites where mean beetle density was greater than 12 beetles m^{-2} (ie. moderate or

high beetle density sites), there was more than a 50% probability of a halo. Halos that were visible at sites in 1999 were still visible in 2000.

The decrease in leafy spurge height from 1997 to 2000 was significantly greater (ANOVA: df=2:10, F=11.25, P=0.007) at sites that had more beetles than at sites with fewer beetles in 2000. There was a 3-fold greater reduction in the mean height of leafy spurge plants at high and moderate beetle density sites over low beetle density sites (Fig. 3.1a).

The reductions in percent cover and stem density of leafy spurge from 1997 to 2000 were significantly greater at sites that had more beetles in 2000 (Fig. 3.1b,c, Tables 3.1 and 3.2). The reduction in mean percent cover of leafy spurge cover was significantly higher at sites with high beetle densities than at sites with low beetle densities (Fig. 3.1b). Similarly, there was approximately a 5-fold greater reduction of leafy spurge stems at high beetle density sites than at low beetle density sites (Fig. 3.1c). Distance from the release point up to 5 m was not an important predictor in changes in leafy spurge cover or stem density (Tables 3.1 and 3.2).

Photographs were taken at each site in 1997, prior to the release of the beetles and in 2000. Decreases in the amount of leafy spurge were visible around the point of beetle release from 1997 to 2000 at some of the sites that had high beetle densities in 2000 (eg. Fig. 3.2). Some of the sites with low beetle densities in 2000 had visible increases in the amount of leafy spurge around the point of release from 1997 to 2000 (eg. Fig. 3.2).

Discussion

Although control sites were not established in this study, it was possible to attribute the decline in weed populations to the density of the biocontrol agent. There were significantly greater decreases in leafy spurge height, percent cover, and stem density at sites that had higher numbers of *A. lacertosa* than at sites with lower beetle densities. Decreases of leafy spurge were often visible during the second and third years post-release of the beetles; the most prominent sign of beetle impact at a site was a halo of dead leafy spurge stems close to the area where the beetles had been released.

In this study, there was no relationship between distance from the beetle release point (up to 5m) and the decrease of leafy spurge. It was expected that the greatest reductions in leafy spurge would be closest to the release point and it was expected that this relationship would be especially prominent at sites where there were halos near the release point. However, halo changes overtime probably obscured this relationship. In 1999, the halos tended to be a circle of dead leafy spurge shoots. In 2000, at many sites, leafy spurge began to re-grow in the center of the halos. These new leafy spurge shoots tended to be short, vegetative suckers and these new shoots were included in percent cover and stem density measurements because there was uncertainty which individual shoots were new. This explains why distance was not a significant factor in explaining the decreases in leafy spurge percent cover and stem density data.

Reductions of leafy spurge increased with beetle densities but were not predictable in space. Although beetles were released in a single location, the beetles did not necessarily move out in concentric circles from the release point. In fact, as evidenced by the halos, the beetles often seemed to move to particular leafy spurge

plants. The flea beetles readily walk, jump, and fly (Harris, 2000) and although there are no data to suggest how far *A. lacertosa* can move in a season, *A. nigriscutis* is capable of traveling up to 200 m (Jonsen *et al.*, 2001). A lot of beetle movement was observed at release sites on the Blood Reserve, Alberta and the beetles often chose to relocate to south-facing slopes or move up draws (personal communication - Monte Thomson and Ray Wilson, Agriculture and Agri-Food Canada, 2000). Further study is required to understand how beetle behaviour affects leafy spurge biocontrol; an initial study of *A. lacertosa* behaviour is presented in Chapter 5.

This study demonstrated that 3 years post-release, higher densities of *A. lacertosa* significantly reduced the amount of leafy spurge when compared to lower densities of the beetles. Although the impact observed so far is in a localized area (ie. within 5 m of the release point), it is predicted that continued impact by the beetles will result in leafy spurge biological control at some sites. Future studies conducted at larger spatial scales should continue to evaluate the efficacy of this biocontrol agent. The changes that occur in halos over time should also be followed.

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Table 3.1 Summary of split-plot ANOVA statistics for the effects of *Aphthona lacertosa* density (low, moderate, or high) and distance (1, 3, or 5 m from the release point) on the average decrease of leafy spurge percent cover.

Source of variation	df	F-ratio	P-value
Density	2	8.396	0.009
Distance	2	2.537	0.107
Density*Distance	4	0.747	0.572
Error	27		

Table 3.2 Summary of split-plot ANOVA statistics for the effects of *A. lacertosa* density (low, moderate, or high) and distance (1, 3, or 5 m from the release point) on the average decrease of leafy spurge stem count.

Source of variation	df	F-ratio	P-value
Density	2	5.876	0.017
Distance	2	0.005	0.995
Density*Distance	4	1.13	0.366
Error	27		

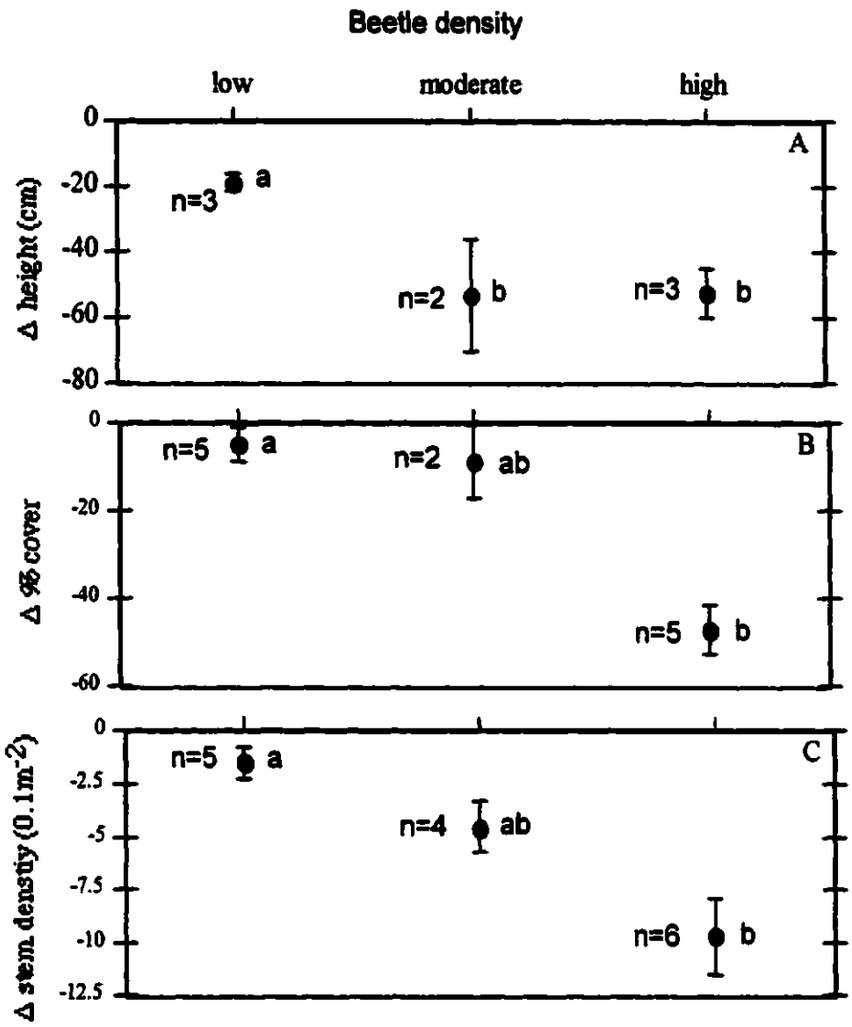

Figure 3.1 Mean change (±SE) in leafy spurge (A) height, (B) percent cover, and (C) stem density from 1997, immediately prior to the release of the biocontrol agent *Aphthona lacertosa*, to 2000. Beetle density classes are based on bi-monthly sampling in 2000 (see Table 2.1). The number of sites per density class is indicated and letters represent significant differences between low, moderate, and high beetle density sites ($\alpha \leq 0.05$).

A: site 111 in 1997

B: site 111 in 2000

C: site 021 in 1997

D: site 021 in 2000

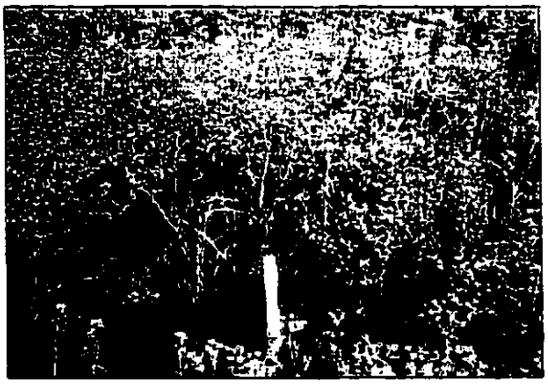

Figure 3.2 Photographs before (1997) and after (2000) the release of the leafy spurge biocontrol agent, Aphthona lacertosa, at a site that had a high beetle density in 2000 (site 111, photos A and B) and at a site that had low beetle density in 2000 (site 021, photos C and D). The yellow plants in the photos are leafy spurge. The red and white sign and the middle white post were the beetle release points in 1997 at sites 111 and 021, respectively.

Chapter 4

Factors affecting the density of a classical weed biocontrol agent

Abstract

It is assumed that successful weed biocontrol requires high densities of the biocontrol agent to top-down regulate the weed population. In 1997, *Aphthona lacertosa* was released for the biocontrol of leafy spurge in Alberta. The purpose of this chapter was to assess if *Aphthona lacertosa* densities were affected by soil composition, food abundance (ie. amount of leafy spurge) at the time of release, beetle size and instantaneous egg load, and/or site cumulative degree-days (CDD). Sites were monitored in 2000 and peak beetle abundance was low at 7 sites (< 10 beetles m^{-2}), moderate (10-70 beetles m^{-2}) at 4 sites, and high (>70 beetles m^{-2}) at 6 sites. Statistical tests were conducted to test if the factors of interest were significantly different between sites with low, moderate, and high beetle densities. Beetle densities in 2000 were independent of soil composition and 1997 food abundance ($P>0.065$). Beetle density was best explained by the number of CDD at a site. Bigger beetles had greater instantaneous egg load ($r^2=0.424$, $P=0.003$). Sites that accumulated more CDD earlier in the season had bigger beetles (for females: $r^2=0.678$, $P=0.001$). Thus, leafy spurge will probably be more quickly reduced at sites that are warmer, such as in southeastern Alberta, because those populations of *A. lacertosa* have the potential for the greatest population growth.

Introduction

Classical weed biocontrol is the introduction of a foreign enemy to control an introduced weed in an ecosystem where the weed has no natural predators (Zwolfer *et al.*,

1976; Huffaker *et al.*, 1971; Bartlett and van den Bosch, 1964). Classical biocontrol has resulted in the successful control of some invasive weeds including the Klamath weed (*Hypericum perforatum* L.) in northwestern United States rangelands, the aquatic alligator weed (*Alternanthera philoxeroides* (Martius) Grisebach), and the floating fern (*Salvinia molesta* D.S. Mitchell) (Crawley, 1989). In these successes, the weed populations were decreased by top-down regulation by "outbreak" densities of the biocontrol agents.

Biocontrol agents should be released in areas where they will be able to attain maximum densities for the best chance of weed control. However, in many cases, there is uncertainty as to how a biocontrol agent will behave in the new ecosystem (Simberloff, 1989; Erlich, 1986) and thus, studies must be conducted to discover what factors affect the biocontrol agent. Predation is usually not a factor for consideration because most classical weed biocontrol agents are free from the species-specific predators that occur in their native habitat (Harris, 1991). Instead, the densities of the herbivores in non-native environments are most likely to be limited by abiotic factors including temperature and humidity extremes (Gassmann *et al.*, 1996), and other environmental factors (Grevstad, 1999).

The purpose of this study was to assess factors that could affect the density of *Aphthona lacertosa* populations, a classical biocontrol agent that was recently (1997) released to control leafy spurge in Alberta. Factors that were considered in this chapter include soil composition, food abundance, beetle size and potential fecundity, and cumulative degree-days. The factors examined in this chapter do not represent an

exhaustive list of the factors that might influence *A. lacertosa* densities but they represent the factors that I predicted were the most likely to have the greatest impacts.

1) Soil composition

Soil composition may play a role in the success of an introduced *A. lacertosa* population because about 9 months of the beetle's lifecycle is spent in the soil. *Aphthona lacertosa* has been reported to have a preference for loamy soils (Gassmann *et al.*, 1996), soils that are composed of 23-52% sand, 28-50% silt, and 7-27% clay (Singer and Munns, 1996). In Europe *A. lacertosa* is also associated with higher levels of clay and organic matter (Nowierski *et al.*, 1996). Thus, it was predicted that sites with loamy soils and higher levels of clay and organic matter would have the highest densities of *A. lacertosa.*

2) Food abundance

It was predicted that the initial amount of leafy spurge within 5 m of the release point could affect the population densities of *A. lacertosa.* This prediction was based on studies that show a relationship between food quantity and insect growth (Barbosa and Schultz, 1987; Slansky and Scriber, 1985; Denno and McClure, 1983). It was hypothesized that population densities would be higher at sites that originally had greater leafy spurge densities and taller leafy spurge plants around the immediate area where the beetles were released.

3) Beetle size and potential fecundity

Beetle densities may be affected by beetle size and fecundity. The more favorable a habitat is, the larger an insect should be able to grow, provided the carrying capacity has not been reached (Begon *et al.*, 1996). It was predicted that *A. lacertosa* would be biggest at the highest density sites, assuming that the sites with the highest beetle densities had the most favorable beetle habitat. It was further hypothesized that the biggest beetles would produce the greatest number of eggs since this relationship has been shown for other insects (Mills and Kuhlmann, 2000; Jervis and Copland, 1996; Honek, 1993). To test these hypotheses, wing lengths were compared across sites with low, moderate, and high beetle densities. Wing length was used as a surrogate of body size because wing length can be more accurately measured than body size and it has been shown that wing length is strongly correlated with body size for insects and birds (Miller, 1997; Rodway, 1997; Lanciani and Le, 1995).

4) Cumulative Degree Days (CDD)

The CDD at a site may affect beetle density since it has been shown that insect development and growth rates increase linearly with temperature (Gilbert and Raworth, 1996; Lanciani and Le, 1995; Dixon *et al.*, 1982). It was predicted that release sites with more CDD would have higher densities of beetles because these sites would have earlier beetle emergence, meaning there would be more time for the beetles to find a mate and choose a suitable location for laying eggs. Although temperature does not directly affect adult beetle growth, temperature is correlated with many other factors that may affect beetle growth over time including the amount of time available to feed (Gilbert and

Raworth, 1996), food quality, and food abundance (Kalischuk *et al.*, 2001). Thus, it was predicted that sites with more CDD would have more, and bigger beetles.

Methods

Beetle and weed density data collection

Aphthona lacertosa were released at 94 sites in Alberta in 1997. Density data were collected on *A. lacertosa* and leafy spurge at 50 and 17 release sites in the summers of 1999 and 2000, respectively, as previously described (see Chapters 2 and 3: Methods). Sites were classified into low (<10 beetles/m^2), moderate (10-70 beetles/m^2) or high (>70 beetles/m^2) density sites as previously described (see Chapter 2: Methods).

Soil and food abundance data collection and analysis

Data were collected on leafy spurge density and soil composition at each site in 1997, prior to the release of *A. lacertosa* by Alberta Agriculture Food and Rural Development. These data were used to determine whether leafy spurge density or soil composition at a site were factors that contributed to beetle population growth.

Two soil samples were collected to a depth of at least 30 cm in the vicinity of the release area in 1997 at all sites. Alberta Research Council, Vegreville, analyzed soil samples to measure the average percent of sand, silt, clay, and organic matter. To test if beetle density was affected by soil composition, a multiple linear regression was conducted (all statistical analyses were performed using SYSTAT, 1998) with the response variable being the mean peak beetle density at a site in 2000 (n=17 sites) tested

against the predictor variables of mean percent clay and organic matter. Since sand and silt are correlated with clay composition, these variables were not included. Mean percent clay and organic matter data were arcsin square-root transformed to ensure homogeneity of variances (Zar, 1999).

To test if beetle population growth was affected by the initial amount of leafy spurge at a site, 3 linear regressions were conducted with the independent variables of mean leafy spurge canopy height, percent cover, or stem density at a site in 1997 tested against the log-transformed 2000 peak beetle density. Beetle density is directly related to beetle population growth; the beetles were the from the same source population and similar numbers of beetles were released at the sites (see Chapter 2: Methods), so sites with higher beetle densities in 2000 had more population growth than sites with lower beetle densities. Canopy height of leafy spurge at sites in 1997 was measured in one location, near the point of release. Mean leafy spurge percent cover and stem density at each site in 1997 were calculated using measurements from 12 quadrats - 3 distances (0.5, 2.5, and 4.5 m from the release point) in the 4 directions (north, south, east and west). Leafy spurge height and stem density measurements from 1997 were missing at 4 and 1 of the 17 sites, respectively.

Wing lengths

Wings were measured using an image analysis system (Kokko *et al.*, 1996). Wings were removed from the beetles using fine tweezers and placed on a glass microscope slide in a drop of water. After soaking for a couple of minutes, the wings were spread flat using a fine bristled paintbrush and left to air dry. Microscope slides

were positioned on a Shotz trans illumination fluorescent light source beneath a Wild Photomakrosko M400 stereomicroscope that was fitted with a Hitachi HV-C20 video camera. The stereomicroscope was set with a magnification of 7X and digital greyscale images were acquired at a pixel resolution of 120.6 pixels/mm. Wing measurements were made using Image Tool for Windows (Version 2, 2001, http://ipt.lpl.arizona.edu/). Both wings from beetles in the 1999 collections were scanned and the subsequent best, intact wing was selected for measurement. For each wing, the length and width was measured (Fig. 4.1). In 2000, only wing length was measured because, based on the 1999 results, there was a good relationship between wing length and wing width (Fig. 4.2). Female and male beetle wings were measured to test if size differences between sites, which might relate to dispersal ability, were similar for both sexes.

The relationship between wing length and the number of eggs from female beetles in peak density samples in 2000 was tested using a linear regression. Beetle sizes were compared across sites with low, moderate, and high beetle density in 2000. To ensure that the beetles were at similar phenological stages of development, only beetles that were collected on the date when peak densities occurred were used. Wing length was tested against the predictor variables sex (male or female) and site beetle density (low, moderate, or high) using an ANOVA. Differences between treatment means were assessed using Tukey's HSD test (α=0.05).

Beetle fecundity

The potential fecundity of *A. lacertosa* cannot be measured by dissecting the beetles because *A. lacertosa* are synovigenic (Harris, 2000). Potential fecundity

estimates of the beetles were based on a single egg count. The frozen beetle samples were thawed in water at room temperature, and then the abdomens of the beetles were dissected and the eggs were counted. Beetle instantaneous egg load was compared across sites with low, moderate, and high beetle densities in 2000 using female beetles from peak density samples in 2000. An ANOVA was conducted with the response variable being the log transformed instantaneous egg load tested against the predictor variable of site beetle density (low, moderate, or high). Differences between treatment means were assessed using Tukey's HSD test (α=0.05).

CDD

To test if Cumulative degree days (CDD) affected population growth at a site, an ANOVA was conducted with the predictor variable being the CDD on Julian date 274 (September 30), 2000 tested against the response of *A. lacertosa* site density in 2000 (low, moderate, and high). Julian date 274 was chosen because it is the end of the season for *A. lacertosa* beetles (Harris, 2000). CDD were calculated for *A. lacertosa* release sites as previously described in Chapter 2. Differences between treatment means were assessed using Tukey's HSD test (α=0.05).

To test if there is a relationship between CDD and beetle size, 2 weighted linear regressions were conducted using the number of beetles sampled per site as the weighting factor. The dependent variables average male or female wing length per site in 2000 were tested against the independent variable, Julian date (2000) at each site when the CDD totaled at least 1230 CDD, the predicted timing for peak beetle densities.

Results

Soil composition

Beetle densities in 2000 were not explained by soil characteristics at the release site. Soils were relatively homogeneous; all sites had relatively sand-silt soils mixed with some clay. The average site was composed of 52% sand (±5.5% SE), 32% silt (±3.7% SE), and 16% clay (±0.2% SE). The average amount of organic matter at a site was 5.8% (±12.7% SE). The percent clay ($df=1,14$, $t=1.032$, $P=0.320$) and organic matter ($df=1,14$ $t=0.408$, $P=0.689$) at a site were not significant predictors of beetle density.

Food abundance

There were no statistically significant effects of initial leafy spurge quantity on 2000 beetle densities (Fig. 4.3). Leafy spurge height in 1997 was more closely related to beetle densities in 2000 than either leafy spurge percent cover or stem density in 1997 (Fig. 4.3). Leafy spurge height was marginally significant ($r^2=0.28$, $P=0.065$). Beetle density in 2000 was independent of leafy spurge cover ($P=0.388$) and stem density ($P=0.364$) in 1997.

Beetle size and fecundity

There were relationships between beetle density, beetle size and potential fecundity. Beetle size was related to instantaneous egg load. Wing length accounted for 42% of the variation in instantaneous egg load (Fig. 4.4). Wing lengths of *A. lacertosa* females were significantly larger than males, averaging about 0.20 mm longer (Table 4.1,

Fig. 4.5a). Beetle densities were related to beetle size. Independent of beetle sex, beetle wing lengths varied significantly across sites with low, moderate, and high beetle densities (Table 4.1, Fig. 4.5b). Beetles at moderate density sites were significantly larger than beetles at low or high-density sites. Beetle densities were related to the instantaneous egg load of the beetles. Sites with moderate beetle densities in 2000 had significantly more eggs per female than sites with low or high beetle densities in 2000 (ANOVA: df=2,16, F=3.678, P=0.049). Fecund females at moderate beetle density sites averaged about 5 more eggs per female than fecund females at low or high beetle density sites (Table 4.2).

CDD

There was a significant relationship between CDD and beetle size. Differences between the Julian dates that sites reached peak beetle abundance accounted for 35% and 68% of the size variance in male and female beetles, respectively (Fig. 4.6). The biggest beetles were at the warmest sites. Mean CDD were significantly different across low, moderate, and high-density *A. lacertosa* sites in 2000 (ANOVA: df=2,13, F=5.510, P=0.018) (Fig. 4.7). Sites with high beetle densities did not have significantly different CDD than sites with low or moderate beetle densities. Sites with moderate beetle densities had significantly higher CDD than sites with low beetle densities.

Discussion

Beetle densities in 2000 were independent of soil type and previous food abundance. Alberta Agriculture Food and Rural development targeted the releases of *A.*

lacertosa to sites with loamy soils based on Gassmann *et al.*'s (1996) findings (Jim Tansey, personal communication). Thus, soil types were relatively homogeneous and this may explain why there was no significant effect of soil type on beetle density. Although leafy spurge height accounted for some of the variance in beetle densities, height alone is not a good predictor of food abundance because height changes over a growing season. The supporting measurements of food abundance, which were leafy spurge percent cover and stem density, show that initial food abundance around the release point had no significant effect on beetle density. By 2000, leafy spurge abundance decreased significantly more at high beetle densities sites than at moderate and low beetle density sites (see Chapter 3). Thus, the change in total food abundance from 1997 to 2000 at the release sites should be investigated as a factor in limiting beetle densities, especially at high beetle density sites.

Of all the factors examined in this chapter, CDD were the most important factor for describing the observed differences in beetle densities. As predicted, CDD were related to beetle size with warmer sites having bigger beetles. Also, as predicted, the biggest beetles had the most eggs. However, contrary to the prediction, moderate beetle density sites, not high beetle density sites, had the most CDD and the biggest, and most fecund female.

There are several reasons that may explain why the biggest beetles were found at moderate beetle density sites. Sites with high densities of beetles in 2000 could be termed as "outbreak" sites (personal observation), and thus, these sites likely surpassed their carrying capacity. This would result in intraspecific competition and the outcome of this competition is smaller sized insects (Begon *et al.*, 1996). Intraspecific competition

probably would not limit beetle growth at low-density sites. Instead, unfavorable habitat probably limits beetle size and population growth. Beetles were the largest at moderate density sites because the sites probably provided suitable habitat where intraspecific competition did not limit beetle growth. Additionally, beetles were the biggest at moderate density sites because moderate density sites had the most CDD.

In general, sites that had higher beetle densities had more CDD; however, the highest density sites did not have the most CDD. Moderate-density sites had the most CDD and this seems to contradict the previous conclusions that higher temperatures resulted in an increased body size, an increased body size resulted in increased egg counts, and therefore, this reasoning suggests that more CDD should have resulted in higher beetle densities. However, Carroll and Quiring (1993) have shown that high temperatures resulted in reduced egg quality and an increase in the production of nonviable eggs in the spruce bud moth. Others have also shown that potential fecundity (number of eggs produced) is not always equivalent to realized fecundity (number of progeny produced) (Mills and Kuhlmann, 2000; Leather, 1988). Thus, even though moderate density sites had the warmest temperatures and the biggest beetles with the highest potential fecundity, the realized fecundity at moderate density sites may be much lower than at low or high-density sites due, in part, to the warm temperatures.

In conclusion, the efficacy of *A. lacertosa* as a biocontrol agent may be improved if the beetles are released at sites that are warmer. In general, sites that have more CDD will have higher densities of *A. lacertosa* than cooler release sites because the warmer sites will have bigger beetles that produce more eggs. Higher densities of *A. lacertosa*

should increase the amount of damage done to leafy spurge plants and result in better leafy spurge biocontrol.

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Table 4.1 Summary of ANOVA statistics for the effects of sex (male or female) and *Aphthona lacertosa* site density (low, moderate, or high) on *A. lacertosa* wing length sampled at peak density.

Source of variation	df	Sum of squares	F-ratio	P-value
Sex	1	1.780	34.359	0.000
Density	2	0.402	7.807	0.000
Sex * Density	2	0.072	1.405	0.247

Table 4.2 Potential fecundity of *Aphthona lacertosa* that were sampled at peak density at sites with low (<10 beetles/m^2), moderate (10-70 beetles/m^2) and high (>70 beetles/m^2) peak beetle densities.

Density	# beetles	mean egg count (± SE)
low	5	14.2 ± 2.56
moderate	6	19.7 ± 1.52
high	8	15.3 ± 0.59

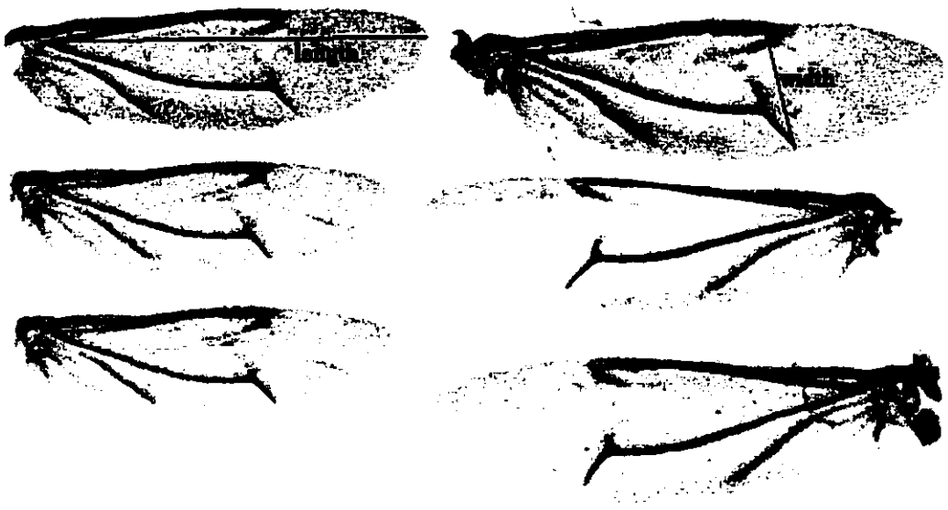

Figure 4.1 Typical wings of *Aphthona lacertosa* male beetles (3 wings on the left) and female beetles (3 wings on the right). Wing length was measured from the vein intersection to the tip of the wing. Wing width was measured from the bottom edge of the top vein to the bottom of the wing at an angle that included the tip of the bottom vein. Scale = 19:1.

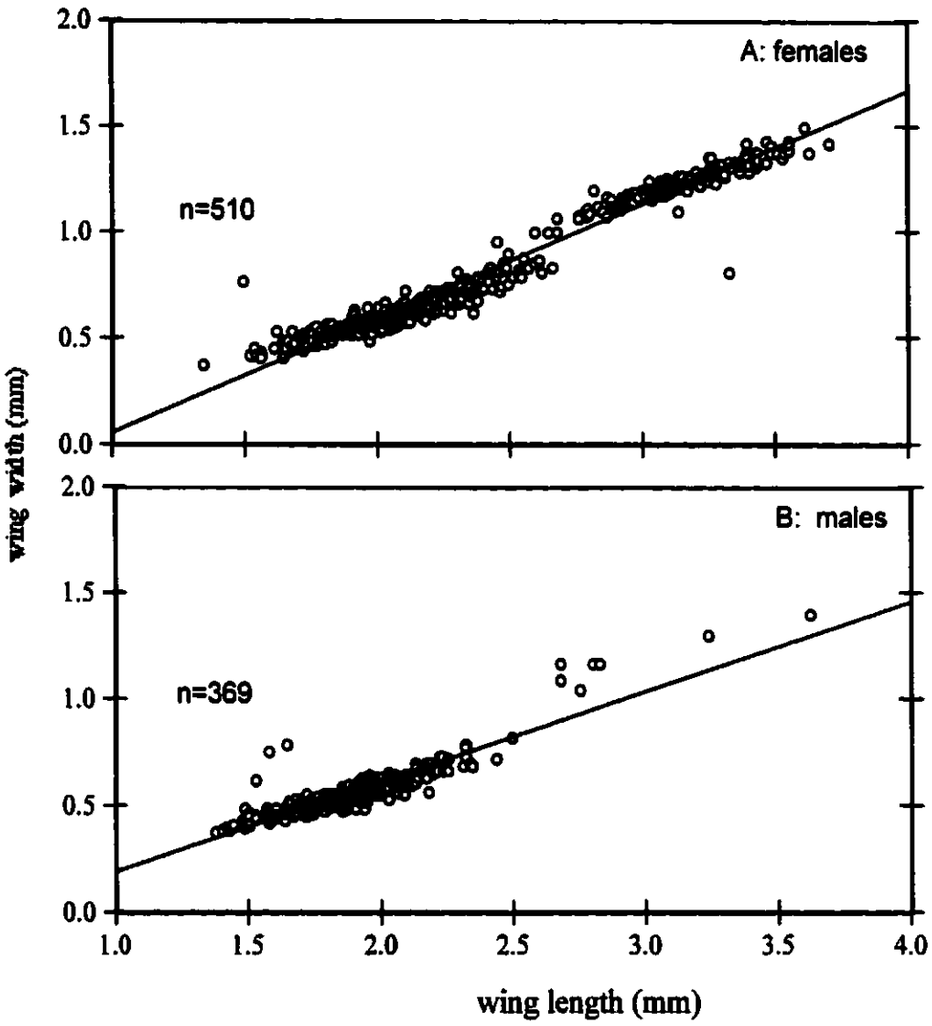

Figure 4.2 Relationship between *Aphthona lacertosa* wing length and wing width for females (A) and males (B) at 50 release sites in Alberta in 1999. The linear regression plot for
females is: width = 0.536(length)-0.472, r^2=0.966 and for
males is: width = 0.424(length)-0.231, r^2=0.826.

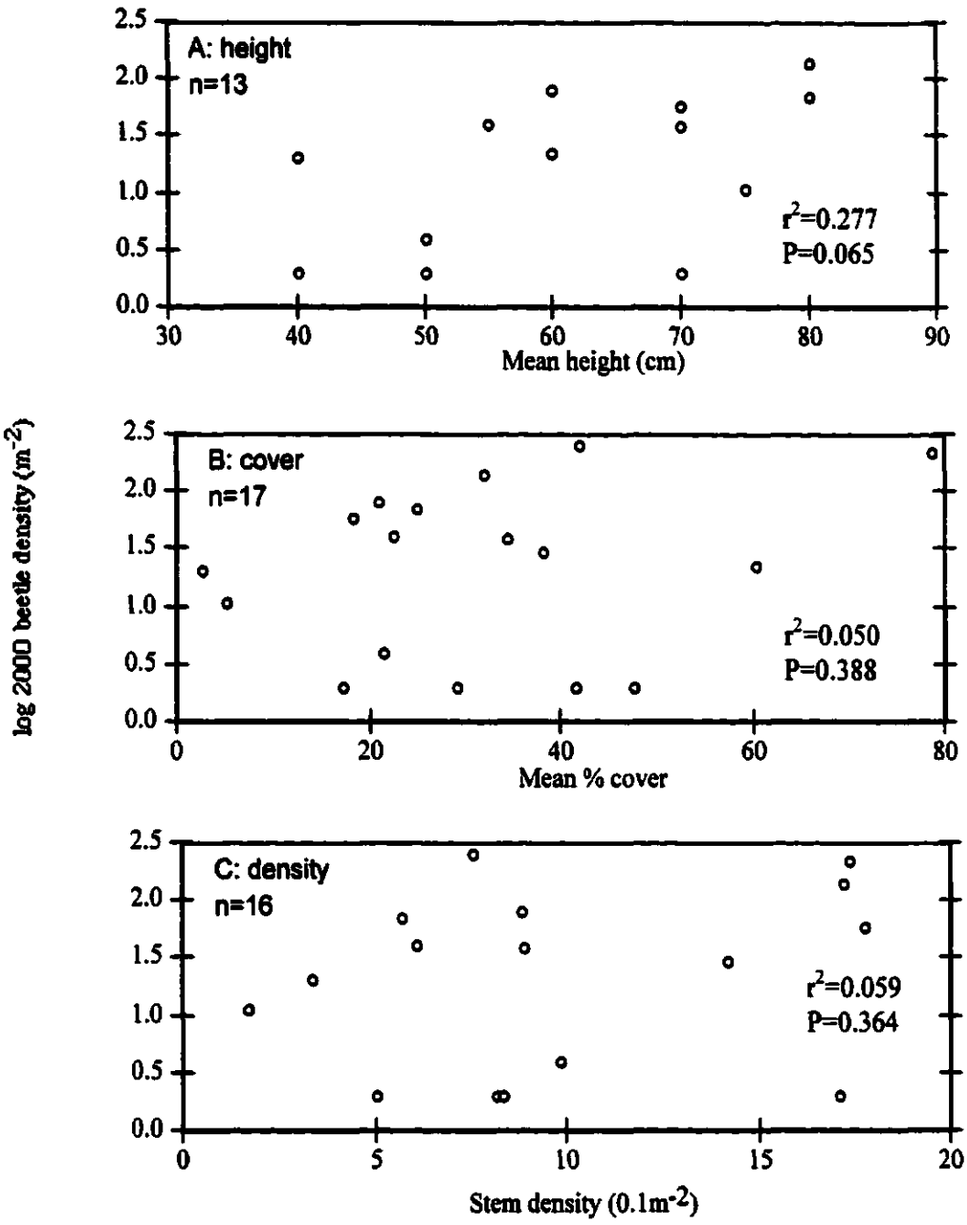

Figure 4.3 Relationship between mean leafy spurge (A) height, (B) percent cover, and (C) stem density in 1997 and *Aphthona lacertosa* densities in 2000 at biocontrol release sites in Alberta.

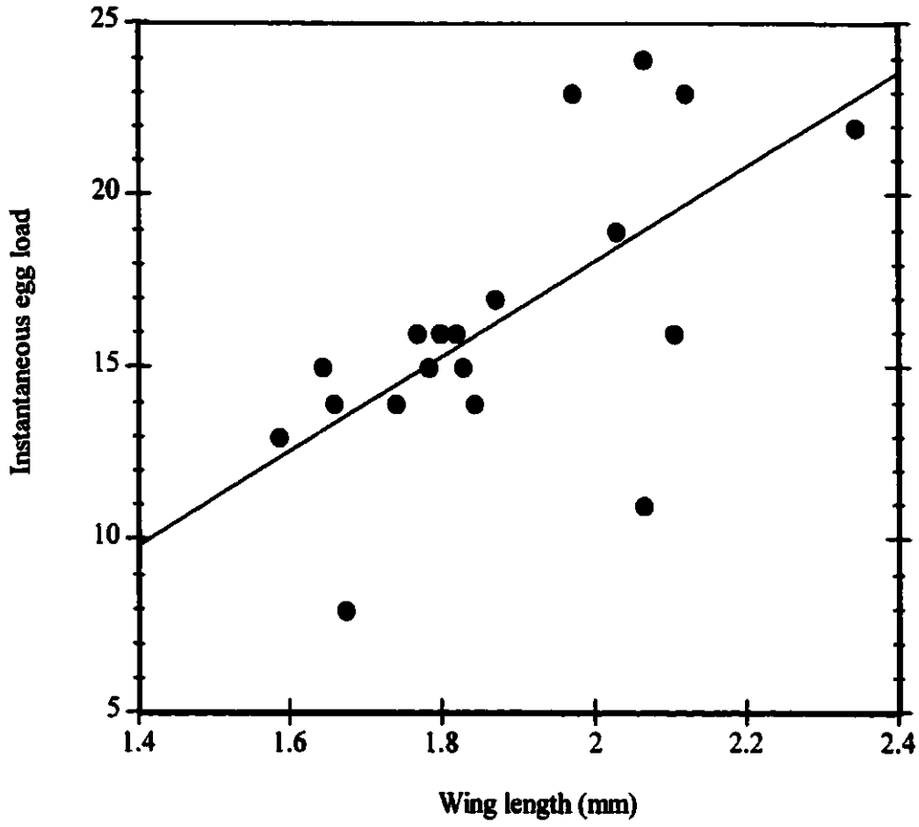

Figure 4.4 Relationship between *Aphthona lacertosa* wing length and instantaneous egg load (n=19) as measured from 8 sites in Alberta in 2000. All data are for beetles at a site when the site density peaked. The linear regression plot is: number of eggs = 13.722(wing length)-9.402, r^2=0.424, P=0.003.

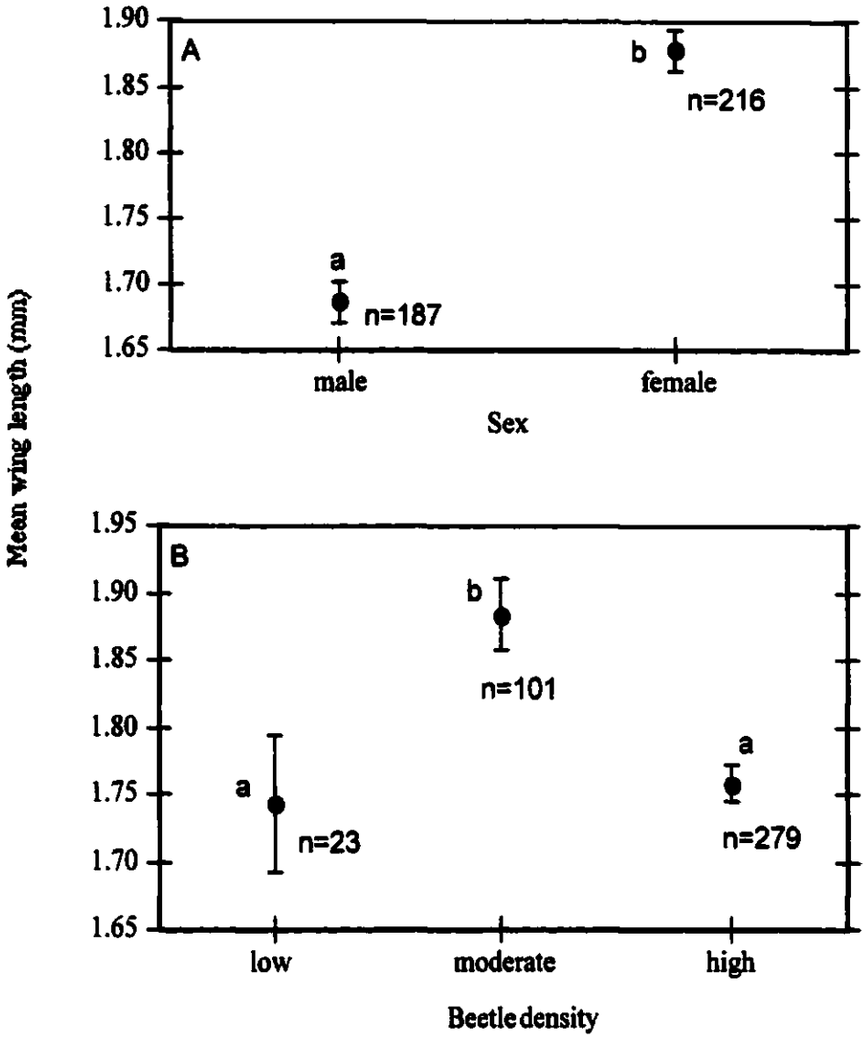

Figure 4.5 Mean wing length (±SE) of *Aphthona lacertosa* male and female beetles (A) at low, moderate, and high density sites (B) in Alberta in 2000 (see Table 2.1 for density classifications). Sites were sampled bi-monthly and only those beetles from peak densities are included. The sample size is indicated and letters represent significant wing length differences ($\alpha \leq 0.05$).

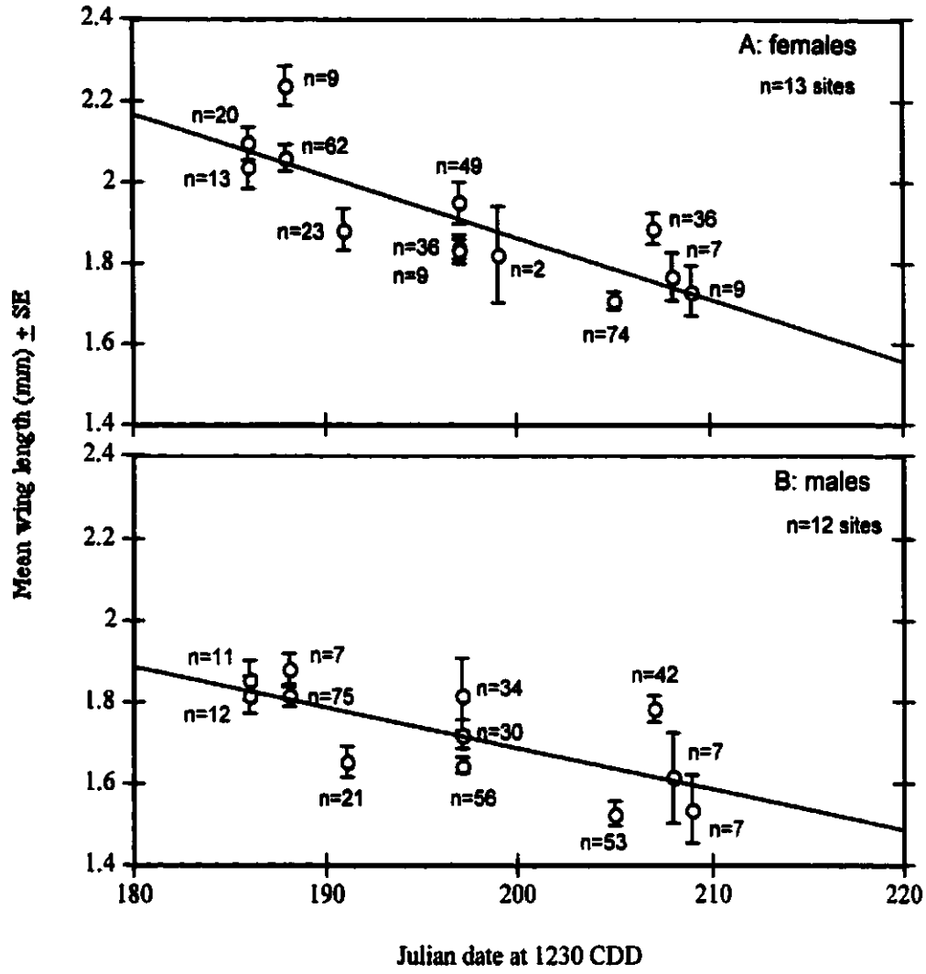

Figure 4.6 Mean wing length of *Aphthona lacertosa* female (A) and male (B) beetles at sites in 2000 and the Julian date in 2000 that each site reached a cumulative degree date (CDD) of 1230, the predicted date for peak beetle abundance. The number of beetle wings measured at each site is indicated. The weighted linear regression plot for:
females is: wing length = -0.016(date)+5.055, r^2=0.678, P=0.001 and for
males is: wing length = -0.009(date)+3.448, r^2=0.352, P=0.042.

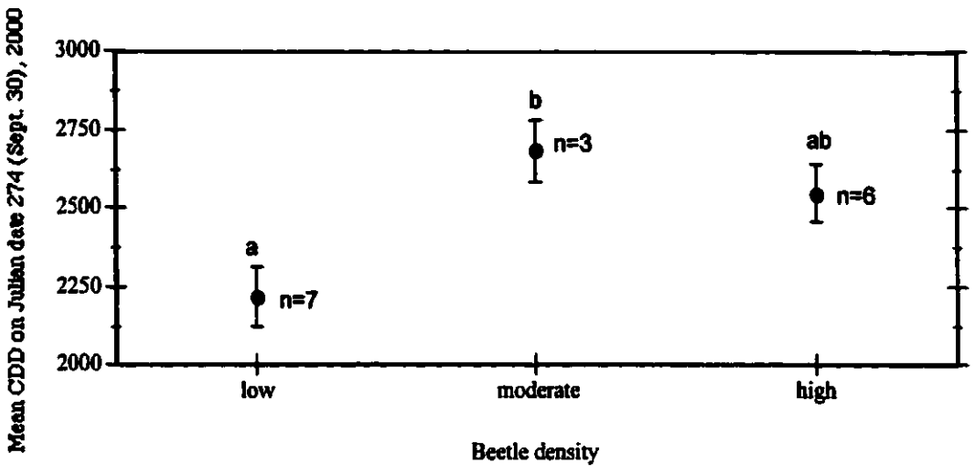

Figure 4.7 Mean cumulative degree days (CDD) (±SE) at the end of September at biocontrol release sites where *Aphthona lacertosa* densities were low, moderate, and high (see Table 2.1 for density classifications). The letters indicate significant differences between site densities ($\alpha \leq 0.05$).

Chapter 5

Host plant characteristics affect beetle distribution and feeding patterns

Abstract

The efficacy of a biocontrol agent may increase if the agent aggregates. The purposes of this chapter were to determine 1) if adult *A. lacertosa* actively aggregate on leafy spurge shoots and 2) if plant morphology affects the distribution and feeding patterns of the beetles. Beetles (n=400 per site) were released in the middle of a patch of 20 flagged leafy spurge shoots at 25 sites in Pavan Park, Lethbridge, AB. Twenty-four hours post-release, the number of *A. lacertosa* and the number of leaves with beetle feeding damage per leafy spurge shoot were counted. Leafy spurge shoot attributes were also measured. The distribution and feeding damage of the beetles were aggregated on individual leafy spurge shoots from both vegetative and flowering populations ($P<0.001$). After 24 hours, beetles were more likely to be found on flowering rather than vegetative shoots ($P=0.011$). Feeding damage was more likely to be found on leafy spurge shoots that were closer to the release point ($P<0.002$), shorter ($P<0.000$), and vegetative ($P<0.002$). This study showed that *A. lacertosa* actively aggregate. This study also showed that plant morphology affects beetle distribution and feeding patterns. More detailed, longer-term studies on the behaviour of *A. lacertosa* will enable better recommendations for the future control of leafy spurge.

Introduction

The efficacy of a biocontrol agent may increase if the agent aggregates (Gassmann, 1996; Lawton, 1985; Murdoch *et al.*, 1984). In instances where individual

biocontrol agents choose to aggregate, the aggregate must contribute to the overall fitness of the individual. Aggregation can be beneficial to the agent because the aggregate may be more efficient at detecting or protecting self from potential predators, have the ability to better detect and use food, and may be able to moderate adverse environmental factors (Romoser and Stoffolano, 1994).

Rosenheim *et al.* (1989) reviews the differences between active and passive aggregation. Active aggregation requires the individuals to choose particular units of the host population. Attractants that result in active aggregation include feces (Wendler and Vlatten, 1993), other individuals (Ishii, 1970), visual or tactile interest (Berthold and Wilson, 1967), and plant compounds (Fegueiras *et al.*, 1994; Sakuma and Fukami, 1990). Passive aggregation does not require individuals to choose particular units of the host population. Instead, passive aggregation results from demographic effects of the host population (Freeman, 1982; Strassman, 1981).

Identification of the factors that affect biocontrol agent aggregation should enable better recommendations for future releases. For example, if the aggregation attractant is a plant compound, it may be possible to manipulate biocontrol agent movement by dispersing the plant compound on problem weed patches. Alternatively, if the aggregation attractant is other biocontrol agent individuals, it may be possible to manipulate damage to host weed plant populations by moving individuals from one problem weed patch to another.

Leafy spurge biocontrol has been attributed to *Aphthona lacertosa* (Chapter 3), an agent that actively aggregates both in its adult and larval forms (Harris, 2000; Gassmann *et al.*, 1996; Gassmann, 1990). It was observed that the adult beetles seemed to aggregate

on particular leafy spurge shoots during 1999 and 2000 monitoring of 1997 *A. lacertosa* release sites. It was common to have a single shoot of leafy spurge heavily attacked by *A. lacertosa* while a neighboring shoot was left untouched at sites with high beetle densities. Often, the heavily attacked shoots were short, vegetative shoots and the untouched shoots were taller and flowering. Although there seems to be consensus that the beetles actively aggregate on leafy spurge stems (Harris, 2000; Gassmann *et al.*, 1996; Gassmann, 1990), it is unknown what causes the aggregation and why the aggregation occurs. If leafy spurge affects beetle behaviour, the beetle distributions on these shoot types could be driven by host plant characteristics such as the differences in plant genotype or by the differences in morphological characteristics (phenotype).

Previous studies have shown that insects are influenced by host plant characteristics. For example, egg and larval survival of the leafy spurge gall midge was strongly influenced by genotype (Lym 1996). Rather than being influenced by genotype, insects may prefer plants or plant parts that are a particular age or size. Murugan and George (1992) found that *Daphnis nerii* (Lepidoptera) prefers to feed on younger leaves rather than mature or senescent leaves. Tinney *et al.* (1998) found that plant size may be important for the cinnabar moth, a biocontrol agent released for ragwort (*Senecio.* sp.). Insects have morphological plant preferences because of chemical constituents in the plants such as water, carbohydrates, nitrogen, secondary metabolites and lipids (Hatcher, 1995; deNooij *et al.* 1992; Murugan, 1992; Scriber 1984).

One of the purposes of this experiment was to test if *A. lacertosa* are actively aggregating on leafy spurge shoots. Additionally, this experiment was designed to examine the morphological characteristics of leafy spurge that were predicted to be

important in explaining 1) the beetle's distribution and 2) the beetle's feeding patterns on leafy spurge shoots. It was predicted that leafy spurge shoot type (vegetative or flowering), and shoot height would affect the beetle's distribution and feeding patterns because the beetles use leafy spurge as a food source, a location for mating, laying eggs, and as a refuge. More beetles and more feeding damage were expected on leafy spurge shoots that provided more food and more protection from extreme heat and wind.

Methods

Pavan Park (112°50'N, 49°47'W), Lethbridge, AB was chosen as the location for experimental releases. Leafy spurge is widely dispersed throughout the riparian area and the predominant native vegetation includes grasses, shrubs and cottonwoods (*Populus* sp.). Prior to this experiment, no *Aphthona lacertosa* had been released within the park.

Twenty-five locations were chosen for beetle releases in an area of approximately 500 x 500 m, under the canopy of a cottonwood forest and within 500 m of the Oldman River. Release locations had similar densities of leafy spurge, equal proportions of flowering and vegetative shoots, and the nearest neighboring release location was at least 50 m away.

Within each release location, 20 leafy spurge shoots were flagged in a circular pattern with approximately an equal number of vegetative and flowering shoots. The 20 shoots were haphazardly selected within a 1.5m circle of the release point, a distance that *A. lacertosa* can easily travel within 24 hours (personal communication - Ian Jonsen, Agriculture and Agri-Food Canada, 2001). Not all available shoots within 1.5m of the release point were flagged. The 20 shoots that were flagged were a subset of all the

shoots within 1.5m of the release point and the shoots within 1.5m of the release point were a subset of all the leafy spurge shoots within the area. Four hundred *A. lacertosa* were released in the middle of each patch of flagged shoots.

Twenty-four hours post-release, the numbers of *A. lacertosa* per flagged shoot (n=500 - 20 shoots*25 releases) were counted. Leafy spurge shoot height, shoot type (flowering or vegetative) and the distance of each shoot from the release point were also recorded. Shoots were then cut at the base and transferred back to the lab where the number of leaves on each shoot were counted and scored as undamaged or damaged by *A. lacertosa* feeding. Feeding damage by *A. lacertosa* is distinct and easily recognizable because this species of flea beetle feeds on leafy spurge by skeletonizing leaf surfaces (Gassmann *et al.*, 1996).

Aggregation analyses

To determine whether *A. lacertosa* and their feeding damage were aggregated 24 hours post-release, the Morisita Index of Dispersion (I_d) was calculated and compared to a chi-square distribution (Krebs, 1999). The Morisita Index defines the probability of two randomly selected beetles or damaged leaves being found on the same stem and is preferable to the variance: mean ratio as a measure of departure from randomness (Hurlbert, 1990). I_d is calculated as:

$$I_d = n\,[\Sigma x^2 - \Sigma x / (\Sigma x)^2 - \Sigma x]$$

where: I_d = Morisita's Index of Dispersion

n = Sample size

Σx = Sum of beetles or damaged leaves per shoot

$\sum x^2$ = Sum of beetles or damaged leaves per shoot squared

Vegetative and flowering shoots were analyzed separately. Two indices were calculated for each shoot type 1) for the number of beetles per shoot at 24 hours post-release and 2) for the number of leaves with feeding damage per shoot.

Beetle distribution analyses

Host plant characteristics that affected beetle distribution were determined using a logistic regression model. The response variable was *A. lacertosa* presence (yes or no) on a shoot 24 hours post-release. The variance in leafy spurge height caused by shoot type was removed because leafy spurge height and shoot type were strongly related (Fig. 5.1). Thus, the predictors were the distance of the leafy spurge shoot from the release point, leafy spurge height residuals (variance in height by shoot type removed) and shoot type (vegetative or flowering). Test statistics were compared to a chi-squared distribution.

Beetle feeding patterns analyses

Feeding preferences of *A. lacertosa* were determined using a Quasi-likelihood regression model with a logit link + (u(1-u)) variance function (McCullagh and Nelder, 1989). The response variable was the proportion of leafy spurge leaves damaged per shoot by *A. lacertosa* 24 hours post-release. The predictors in this model were the same as the predictors in the preceding analysis: the distance of the leafy spurge shoot from the release point, leafy spurge height residuals (variance in height by shoot type removed)

and shoot type (vegetative or flowering). Test statistics were compared to an F distribution.

Results

Four percent of the released *A. lacertosa* were recovered on the 500 leafy spurge shoots 24 hours following their release. Both the flea beetles and their feeding damage were aggregated on individual leafy spurge shoots from both vegetative and flowering populations (Table 5.1).

After 24 hours, *A. lacertosa* were more likely to be found on leafy spurge shoots that were flowering rather than vegetative (Table 5.2). Flowering shoots (n=260) had an average of 1.11 beetles (±0.185 SE) on each shoot in comparison to vegetative shoots (n=240) that had an average of 0.54 beetles (±0.120 SE) on each shoot.

Feeding damage was more likely to accumulate on leafy spurge shoots that were vegetative, shorter, and closer to the release point (Table 5.3). The mean percentage of leaves with feeding damage on flowering shoots was 3.8% (±0.4 SE) and on vegetative shoots was 6.1% (±0.7 SE).

Discussion

This study showed that *A. lacertosa* and their feeding damage are aggregated on individual leafy spurge shoots, both vegetative and flowering, 24 hours post-release. Although leafy spurge shoots may have been clumped within a release patch, the shoots' distribution would probably not account for the aggregation of the beetles or their feeding because all of the shoots were within easy travel distance of the beetles. Thus, any of the

shoots examined in this study should have had an equal opportunity of being found by *A. lacertosa* and in fact, distance was not a significant factor in explaining beetle distribution (Table 5.2).

The reasons for beetle aggregation or the mechanisms that cause the beetles to aggregate are not certain. However, it is suspected that *A. lacertosa* actively aggregate in response to pheromones emitted by other individuals of their species (personal observation and Jim Tansey, personal communication). *Aphthona lacertosa* aggregation may be beneficial to the beetles for the purpose of overcoming leafy spurge defenses. The beetle aggregates may reduce the turgor pressure within leafy spurge shoots and subsequently reduce the pressure and flow of the antiherbivorous latex (person communication - Peter Harris, Agriculture and Agri-Food Canada, 1999). It will be important to determine what factors contribute to *A. lacertosa* aggregation to improve the biocontrol practitioner's ability to predict the beetle's behaviour and improve biocontrol efforts.

This study also suggests that *A. lacertosa* distribution and feeding preferences are affected by the morphological characteristics of individual leafy spurge shoots. The distribution of *A. lacertosa* was affected by shoot type and the beetles tended to be found on flowering shoots (Table 5.2). Differences in beetle distributions on flowering and vegetative shoots may be explained by Feeny's hypothesis (1976) that plants that are more apparent in size and growth form are "bound to be found" by herbivores. Flowering shoots, because they tend to be larger than vegetative shoots, may be more conspicuous to flea beetles that are searching for food and shelter.

Aphthona lacertosa tended to feed on shoots that were vegetative, taller and closer to where they were released (Table 5.3). Although shoot distance from the point of release did not affect the beetle distribution, it would be expected that beetles would have spent a longer amount of time on shoots that were closer to the point of release and therefore, the beetles probably had more time to feed on these shoots. Beetles tended to feed on taller shoots, probably because taller shoots would have provided more food than shorter shoots. The feeding preferences of *A. lacertosa* for vegetative shoots are probably related to plant quality. Vegetative shoots tend to be younger than flowering shoots and the younger leaves are easier to digest with higher nutrient and water concentrations (Scriber and Feeny, 1979). Cates (1980) found that monophagous insects prefer to feed on young leaves that are, in general, more nutritious.

It was expected that the beetles would be distributed on leafy spurge shoots that they feed on. Therefore, it is uncertain why the beetles were more likely to be found on flowering shoots but the beetles were more likely to feed on vegetative shoots. The beetles may use the shoot types for different purposes. For example, the beetles have a preference for feeding on vegetative shoots but may prefer to seek shelter, mate, or oviposit on flowering shoots.

The data in this study present a snapshot of *A. lacertosa* behaviour after only 24 hours and should not be interpreted too broadly. However, further studies should be conducted to discover the long-term implications of plant morphology on beetle behaviour. Knowledge of the morphological characteristics of leafy spurge that affect the distribution and feeding patterns of *A. lacertosa* on leafy spurge shoots will enable better recommendations for the future control of leafy spurge.

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Table 5.1 Morisita Index (Id) for *Aphthona lacertosa* distribution and the number of leaves with feeding damage per shoot on vegetative and flowering leafy spurge shoots.

Plant type	Df	Variable	Id	X^2	P
vegetative	239	beetles	10.91	1517.4	<0.001
		feeding	3.95	2793.7	<0.001
flowering	259	beetles	7.37	2087.2	<0.001
		feeding	4.09	3574.6	<0.001

Table 5.2 Analysis of deviance table from a logistic regression model. The response variable is *A. lacertosa* presence or absence on leafy spurge shoots 24 hours post release.

Term	Df	Deviance	P (X^2)	Coefficient Direction
null	499	560.1		
distance	1	1.6	0.200	
height residuals	1	1.3	0.263	
shoot type (veg. or flo.)	1	6.4	0.011	veg<flo
distance*height	1	1.4	0.232	
distance*type	1	0.9	0.346	
type*height	1	2.4	0.125	
distance*height*type	1	0.3	0.585	
residual	492	545.8		

Table 5.3 Analysis of deviance table from a Quasi-likelihood regression model. The response variable is the proportion of leafy spurge leaves damaged per shoot by *A. lacertosa* feeding 24 hours post-release.

Term	Df	Deviance	P (F)	Coefficient Direction
null	499	4525.8		
distance	1	110	0.002	-ve
height residuals	1	256.1	0.000	-ve
shoot type (veg. or flo.)	1	110.3	0.002	veg>flo
distance*height	1	6.4	0.461	
distance*type	1	23.6	0.155	
type*height	1	538	0.480	
distance*height*type	1	0.1	0.937	
residual	492	4013.5		

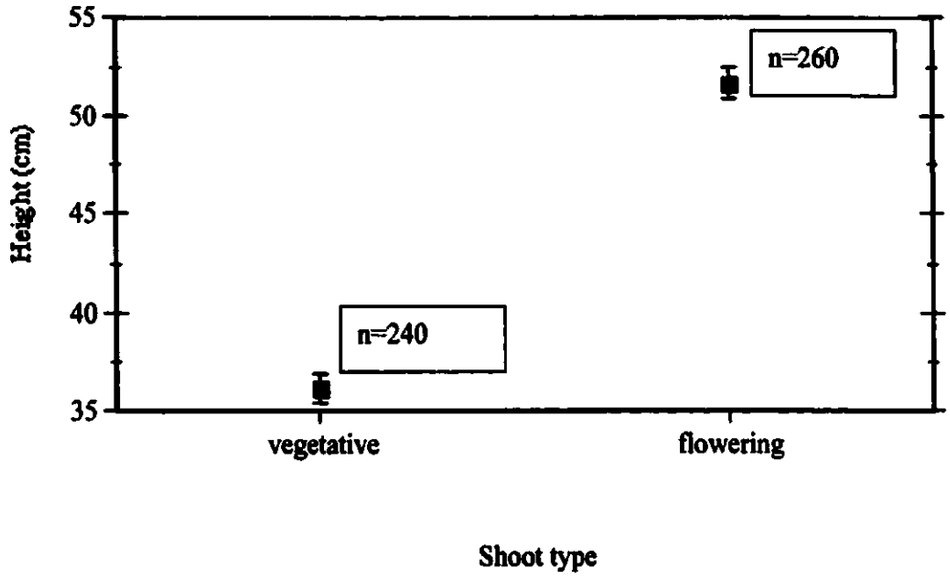

Figure 5.1 Mean height (± SE) of leafy spurge shoots at Pavan Park, Lethbridge, Alberta. Shoot height is significantly different between vegetative and flowering shoots (T-test: t=13.7, df=498, P<0.000).

CHAPTER 6

General conclusions

Mixed *Aphthona lacertosa* and *A. czwalinae* populations were released for the biocontrol of leafy spurge at sites throughout Alberta in 1997. By 1999 and 2000, beetle populations were primarily *A. lacertosa* with *A. czwalinae* contributing to less than 1% of the total number of individuals sampled in 1999 and 2000. *Aphthona lacertosa* established at more than 75% of the 50 release sites that were monitored a single time in 1999. In 2000, beetles were sampled bi-monthly at a subset of 17 of the 50 sites that were monitored in 1999, and beetles were found at all 17 sites. The mean peak beetle density was 126 beetles m^{-2} ($\pm$39 SE) at high beetle density sites in 2000.

Sites that had high densities of *A. lacertosa* had a significantly greater reduction in leafy spurge compared to release sites that had low or moderate beetle densities. Sites with more beetles had significantly greater reductions in leafy spurge canopy height, percent cover, and stem density from 1997 to 2000. High beetle density sites also tended to have a dead zone of leafy spurge around the point of release, a halo, which was directly attributed to the beetles as evidence of impact.

Site attributes were examined to predict what factors affected *A. lacertosa* densities. Beetle densities were independent of soil type and food quantity in 1997 at the 17 release sites sampled in 2000. However, beetle densities were related to the number of degree-days accumulated in 2000 at a site. Sites with more cumulative degree-days had higher beetle densities than cooler sites because the warmer sites had bigger beetles that produced more eggs.

The effects of leafy spurge morphology on *A. lacertosa* were examined. It was

found that the beetle's distribution and their feeding damage were aggregated on particular shoots of leafy spurge. Preferences were based on leafy spurge shoot characteristics including shoot height, shoot type (flowering or vegetative) and the distance of the shoot from the release point. Although broadly applicable conclusions could not be made about the plant preferences of *A. lacertosa*, plant morphology was identified as a potentially important area of study that could affect a biocontrol practitioner's success in controlling leafy spurge with *A. lacertosa.*

Based on the studies in this thesis, it is concluded that future releases of *Aphthona lacertosa* will successfully establish at most release sites in Alberta. Despite their establishment, not all beetle populations will grow large enough to have a significant, negative impact on leafy spurge. To maximize beetle population growth, *A. lacertosa* should be released at sites that tend to be warmer such as in the southeastern parts of the province. Once the beetles have established and reached a high density, it is expected that *A. lacertosa* will effectively control leafy spurge populations.

Implications for leafy spurge control

The best way to control leafy spurge may be with integrated pest management (IPM) using *A. lacertosa* in combination with herbicides, mowing, and/or sheep. This study showed that the beetles are effective at reducing leafy spurge in localized areas; successful biocontrol may take only a few years in southeastern Alberta, where the warm temperatures contribute to faster beetle population growth. However, the few years that it takes for beetles to cause a reduction in leafy spurge may be too long for ranchers and farmers that require an immediate solution to their weed problem. Further, leafy spurge

biocontrol is not a cure-all solution; the beetles will reduce leafy spurge but not eliminate it.

Ideally, a biocontrol release will result in populations of leafy spurge and *A. lacertosa* that exist in a cycle of "mutual rhythmical interchange" (personal communication - Ruth Grant-Kalischuk, University of Lethbridge, 2000). *Aphthona lacertosa* populations will build to a crescendo and deplete leafy spurge populations, and then, there will be a decline in the densities of the *A. lacertosa* populations. The beetles will remain in small numbers until the leafy spurge begins to grow again and then the cycle would repeat. However, IPM should be considered in the cycle because new or small re-infestations of leafy spurge may be best controlled with herbicides. IPM should also be considered if the leafy spurge grows in a dense, large stands. Leafy spurge may best be controlled if beetles are released within the stand and the edges of the stand are sprayed with herbicide to prevent the leafy spurge patch from growing and spreading seed. Also, mowing, sheep grazing, and patchy herbicide application at beetle release sites may augment the control success of A. *lacertosa* because damaged leafy spurge plants will probably be more susceptible to herbivory. The goal of IPM would be similar to the goal of releasing multiple biocontrol agents on a weed - to cause cumulative stress on the weed and provide overall better control than a single control approach (Harris, 1985). Future studies should investigate the options available by integrating biocontrol with other control options because IPM may provide the quickest, most efficient way to get rid of leafy spurge.

Implications for weed biocontrol

The moral of the lessons learned from leafy spurge biocontrol programs is that biological control success requires persistence and patience. These lessons are both encouraging and frustrating for biocontrol practitioners and researchers. Leafy spurge is an example of a weed that has a long history in weed biocontrol. Leafy spurge was introduced into North America in the early 1800's (Gassman *et al.*, 1996) and biological control for the weed began in 1960 (Harris *et al.*, 1985). Over the last 30 years, 18 insects were released in Canada for leafy spurge biocontrol (Julien and Griffiths, 1998) but only 2 have been effective at reducing leafy spurge in some habitats; *A. nigriscutis* and *A. lacertosa*. Since both of these biocontrol agents were recognized (although not formally documented) as "good agents" for leafy spurge biocontrol, biocontrol researchers were encouraged that the root-feeding beetles were the best option for controlling leafy spurge. Thus, a petition was submitted in 1996 for the release of *A. venustula*, another European root-feeding beetle. However, the petition for releasing *A. venustula* was denied. The denial was probably the consequence of the recent concerns that have been raised about the non-target effects of biocontrol agents (Pearson *et al.*, 2000; Louda *et al.*, 1997; Strong, 1997). It was requested that additional non-target host screening be conducted on *A. venustula* to ensure that the threats to existing vegetation are minimal or nonexistent (Bourchier *et al.*, 2001). Non-target host screening is costly and difficult because of the logistics involved in trying to obtain and cultivate species of concern (Harris, 1991). The onus is on biocontrol practitioners to provide proof that the problems caused by the weed far exceed the costs, both environmentally and economically, that may be associated with unpredictable non-target effects, which is a time-consuming and frustrating process.

Nonetheless, biocontrol programs continue because of the economic and environmental benefits. Biocontrol can offer a permanent solution to control a weed problem because biocontrol agents are capable of reproducing and self-dispersing. Also, biocontrol is an appealing "green" alternative for environmentally sensitive areas where herbicide or chemical control is discouraged. Biocontrol agents for weeds like knapweed, hound's-tongue, and leafy spurge continue to be spread to new release sites in Alberta and British Columbia. Similar to *A. lacertosa*, the densities and efficacy of many of the biocontrol agents that are being redistributed have never been evaluated. More post-hoc monitoring studies, like this thesis study, are critical to show that biocontrol agents are behaving as biocontrol practitioners predicted in reducing the weed populations and further studies are needed to investigate possible biocontrol impacts on non-target species.

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