ECOLOGICAL INTERACTIONS OF BIOLOGICAL CONTROL AGENT, *Mecinus janthinus* Germar, AND ITS TARGET HOST, *Linaria dalmatica* (L.) Mill.

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DEDICATION

"In the place my wonder comes from

There I find you

Your face shines in my sky"

(Bruce Cockburn, 1971)

To Bob –

You taught me so much during your brief time here – you are truly an old soul. The kitties and I miss you daily.

And to Mom –

You have a quiet strength that I only wish I had. Your voice on the other end of the phone has kept me sane on more than one occasion. And no, I haven't found a job back in Ontario yet!

ABSTRACT

There has been little documentation of the success of introduced agents for classical weed biological control. Field evaluation of an insect's establishment, spread and early host impact within its new environment must be performed before agent success can either be documented or predicted. Population attributes of the endophagous biological control agent, *Mecinus janthinus* Germar (Coleoptera: Curculionidae), and interactions with its target weed, Dalmatian toadflax, (*Linaria dalmatica* (L.) Mill.) (Scrophulariaceae), were explored across variable levels of resource availability and insect abundance. Patterns of population growth and impact of this biocontrol agent were very consistent throughout this study. Within four years of release, populations of *M. janthinus* achieved outbreak population levels and virtually eliminated the seed producing shoots from toadflax stands. There is a tight but flexible relationship between oviposition site selection and offspring performance in this endophagous herbivore, maximizing offspring survival even under moderate to high *M. janthinus* densities. These attributes allow *M. janthinus* to be an effective biocontrol agent under changing levels of resource availability.

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Introduction and Study Rationale

Classical weed biocontrol

Classical weed biological control involves the importation and release of exotic

biological control agents (i.e. insects or pathogens) to reduce the abundance of target

weeds to acceptable threshold levels (De Clerck-Floate and Bourchier, 2000). The

expectation of biocontrol is that agents will establish in their new environment, making

further releases unnecessary (McFadyen, 1998). Insect biocontrol agents, once

successfully established, can provide continued and cost-effective control of target weeds

(Crawley, 1989). There are examples of successful control of invasive plants by

introduced insects across a range of host types, including the aquatic plant *Salvinia*

molesta (Room *et al.*, 1981), tree *Sesbania punicea* (Hoffman and Moran, 1998), and

perennial weed *Senecio jacobaea* (McEvoy *et al.*, 1991). However, only about 1/3 of

agents released worldwide are successful in establishing and controlling targeted weeds

(Crawley, 1990; Williamson and Fitter, 1996). So how does one determine whether a

biological control agent will establish, successfully achieve and sustain control over its

host plant?

Biological control represents an unique arena in which to study the principles of insect-

plant interactions. Crawley (1986*a*) described the introductions of plants and animals into

new habitats as "some of the most important field experiments ever carried out in

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ecology". The basis for biological control is population ecology; an understanding of the influences on an agent's population establishment, growth and spread is required before their success can be predicted (De Clerck-Floate and Bourchier, 2000). Field evaluation of an insect's population dynamics and interaction with its host within its new environment are key to maximizing its potential impact (De Clerck-Floate and Bourchier, 2000; De Clerck-Floate and Miller, 2002; McClay, 1995).

Biological control of Dalmatian toadflax

Dalmatian toadflax, *Linaria dalmatica* (L.) Mill. (Scrophulariaceae), is an aggressive and highly competitive weed originating from Southeastern Europe – Serbia, Moldavia and Romania (Vujnovic and Wein, 1997). There are no reports of Dalmatian toadflax being a troublesome weed in Europe (Alex, 1962). This attractive plant, originally introduced into North America in 1894 as a showy ornamental (Alex, 1962), has since infested rangelands (Robocker, 1974), open forests and rights-of-way in western North America (De Clerck-Floate and Harris, 2002). Within Canada, toadflax has spread over thousands of hectares of range and forest lands within the Southern interior of British Columbia (BC) and Southwest Alberta (De Clerck-Floate and Harris, 2002). Similar to other invasive plant species (Crawley, 1986*b*), toadflax forms dense, monospecific stands and out-competes native vegetation. Under high densities of *L. dalmatica*, losses in rangeland production can be sustained, as valuable forage becomes displaced by this unpalatable weed (Lajeunesse *et al.*, 1993; Lange, 1958). A highly competitive, stress-tolerant plant (Grime, 1977), toadflax is difficult to manage (Grubb *et al.*, 2002) and has been a target

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for biological control since the 1960's (De Clerck-Floate and Richards, 1997). All species of *Linaria* are classified as primary noxious weeds under the Canada Seed Act, and both Dalmatian and yellow toadflax are schedule A noxious weeds under the Regulation of Weed Control Act in BC (Jeanneret and Schroeder, 1991). Cattlemen in BC consider this weed their third control priority after the knapweeds and houndstongue. (De Clerck-Floate and Harris, 2002).

L. dalmatica is a hemicryptophyte (i.e. most above-ground structures die in the fall), herbaceous perennial (Lajeunesse *et al.*, 1993) with an "exceptional ability to establish by seed" (Vujnovic and Wein, 1997). A single, large plant has been estimated to produce 400,000 (Lange, 1958) to half a million seeds in one growing season (Robocker, 1970). Once established, toadflax also spreads vegetatively by lateral shoots and adventitious root buds (Vujnovic and Wein, 1997). Each vegetative root bud has the capacity to produce a new and independent plant (Lajeunesse *et al.*, 1993). Widely spreading horizontal roots up to 10 feet from the parent plant (Lajeunesse *et al.*, 1993), combined with a deeply penetrating taproot (Alex, 1962), allow established plants to suppress other vegetation by intense competition for limited soil moisture (Robocker, 1974). Annual extension of lateral roots and seed production, combined with a long-lived seed bank, permit a stand of toadflax to persist indefinitely (Robocker, 1974). This combined asexual and sexual reproduction strategy allows Dalmatian toadflax to adapt and reproduce in diverse environmental conditions (Lajeunesse *et al.*, 1993).

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Mecinus janthinus Germar (Coleoptera: Curculionidae), an univoltine, stem-boring specialist herbivore on *Linaria spp.*, was introduced from Southern Europe and Southern Russia into Canada in 1991 for the control of Dalmatian toadflax (McClay and De Clerck-Floate, 2002). The biology of this weevil has been well documented by McClay and De Clerck-Floate (2002), De Clerck-Floate and Harris (2002) for Canada, and Jeanneret and Schroeder (1992) for Europe. Female *M. janthinus* are synovigenic, laying eggs singly into toadflax shoots (Jeanneret and Schroeder, 1992) from early May to late June in Southern BC. During oviposition, females chew holes into toadflax stems prior to depositing an egg. Each oviposition site is covered by the female with a lid (Jeanneret and Schroeder, 1992) and can be readily observed without magnification. Numerous eggs (typically 2-100) are laid per toadflax shoot (De Clerck-Floate and Miller, 2002), resulting in multiple larvae developing within individual stems. Upon hatching, *M. janthinus* larvae construct distinct tunnels of 1-3 cm in length through shoots as they feed (Saner *et al.*, 1994). Development through three larval instars takes place near the oviposition site, followed by pupation within the larval tunnels in mid-summer. Weevils complete their development to adulthood in late August and remain in the shoots until the following spring. Larval tunnels can be easily identified and traced by dissecting the toadflax stems. It has been discovered that *M. janthinus* larval densities within toadflax shoots can be reliably determined in shoot material collected post-weevil emergence (De Clerck-Floate and Miller, 2002).

Beetles have been documented to be the most successful introduced biocontrol agents (Crawley, 1989; Syrett *et al.*, 1996). During the initial screening of *M. janthinus*, this

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shoot-borer was expected to have a greater impact on its host than either defoliators or seed-feeders previously released as toadflax biocontrol agents (Jeanneret and Schroeder, 1991). Both adult and larval feeding play a role in the damage incurred by toadflax plants. Larval boring within stems causes structural weakness, resulting in premature wilting and potential shoot death (Jeanneret and Schroeder, 1992). Short-term studies also indicated that attack by *M. janthinus* larvae significantly reduced toadflax shoot biomass (Saner *et al.*, 1994). The study by Saner *et al.* (1994) suggested that a rate of one pair of *M. janthinus* per toadflax plant should decrease plant vigour and cause late-season shoot and plant mortality. Data from 1994 weevil releases made throughout BC showed that 100% toadflax shoot attack by *M. janthinus* can occur within 3 years of initial release (De Clerck-Floate and Miller, 2002), causing complete suppression of flowering and severe stunting of shoots.

Study location

All of the seventeen sample sites chosen for this study (Table 1.1) were located within the same general climatic and geographic areas: no more than 20 km from the British Columbia – United States border (N 49.0-49.1°), extending west from Christina Lake (W 118.2°) to Rock Creek (W 119.0°) in the West Kootenay region of BC. Each site was separated from others by at least one kilometre within a 95 km east-west corridor. All of the sites were located within an elevation range of 525-950 m, the majority on south to west-facing slopes with 20-80% inclines. Releases of *M. janthinus* were made on large, dense stands of Dalmatian toadflax (400 m to >1 ha in area with densities of more than

6 plants/m^2) growing on coarse textured-soil slopes. Study sites were characterized by a dry, warm climate with average growing season precipitation of 136-169 mm, and a range of growing season temperatures from 9.7-18.5°C (Lloyd *et al.*, 1990). Vegetation consisted of open pasture, parkland grasses and shrubs interspersed with stands of Ponderosa pine and Douglas fir. A single *M. janthinus* release of between 200 and 600 individuals had been made at each of the 17 sites used in this study by the BC Ministry of Forests. Releases were made between 1994 and 2001 from previously established, successful release sites in Kamloops and Grand Forks, BC (De Clerck-Floate and Harris, 2002).

It is important to note that extensive work has been done since 1991 to distribute and establish *M. janthinus* onto toadflax patches throughout the Southern interior of BC. There also has been some natural dispersal of the weevils to new patches. As a consequence, all of the sites chosen for this study had some level of weevil establishment and quite variable *M. janthinus* colonization histories (i.e. differences in time since establishment, original size of weevil release, rate of population increase). The release sites, themselves, varied in original toadflax patch size, plant density and habitat characteristics (i.e. slope directions and degrees, elevations, biogeoclimatic zones). To address this variability, sites were chosen and categorized based on 2001 estimates of weevil population abundance at the original point of weevil release. The methods used to determine *M. janthinus* population abundance are, therefore, addressed in this introduction.

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Adult *M. janthinus* abundance can be reliably estimated using a technique for counting

emerged adults in the field (R. A. De Clerck-Floate, unpublished). The count data have

been found to correlate well with the density of adults of the same generation dissected

from toadflax stalks prior to spring emergence (R. A. De Clerck-Floate, pers. comm.).

Adult weevil counts also can be used to reliably predict the population of offspring

reaching adulthood within the same season (Fig. 1.1). The technique consists of observers

walking at a steady pace while visually inspecting Dalmatian toadflax shoots within 30 m

of the point of original weevil release, and counting the adult weevils seen on stems and

leaves during a 5-minute period. Adult *M. janthinus* are relatively large (3-5 mm in

length), conspicuous black insects against a backdrop of green host plant tissues, and can

easily be seen without disturbing the surrounding toadflax shoots. Two observers

performed the counts in each sample year, after taking repeated practice counts to ensure

the standardization of data collection. Counts made at study sites were consistent between

observers in both years (2001: $t_{pooled}=-0.290$, $P=0.774$, 2002: $t_{pooled}=0.925$, $P=0.358$).

Each observer made a single count per study site in 2001, and two counts apiece in 2002.

The mean counts per minute at each of the 17 study sites are recorded in Table 1.1.

One central theme to this study is the comparison of *M. janthinus* characteristics and

behaviour, as well as host impact, under varying levels of weevil density. Study sites

were classified as "non-outbreak" and "outbreak" according to *M. janthinus* population

estimates. Weevil release sites designated as "non-outbreak" were those most recently

established (1999-2001), with zero to low levels of host attack and <20 adult weevils

counted per minute, whereas those referred to as outbreak sites supported large

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populations of *M. janthinus* (>20 weevils counted per minute), attacking host shoots since 1994-1998 (Table 1.1).

Study rationale

The success of a biological control agent largely depends on three variables (McFadyen, 1998). The first involves assessing the damage that individual biocontrol agents can inflict on target plants. Chapter 2 of this study addresses the significance of adult *M. janthinus* feeding to Dalmatian toadflax individual plants and populations. Previous work by Jeanneret and Schroeder (1991, 1992) and Saner *et al.* (1994) has determined that feeding damage by *M. janthinus* larvae within toadflax shoots causes reduced reproductive output and plant vigour. This study addresses the previously unexamined contribution of adult *M. janthinus* feeding on the sexual reproductive potential and above-ground shoot production of Dalmatian toadflax plants.

To fully understand the impact of herbivory on target weeds, one must also evaluate the ecology of the host, which in turn, determines whether the damage caused by a particular agent is significant in reducing the population (Cullen, 1995). Several studies have addressed the growth, establishment and resource allocation patterns of *L. dalmatica* (Robocker, 1970, 1974; Robocker *et al.*, 1972). This study (Chapter 2) indirectly addresses toadflax reproduction and above-ground vegetative growth under different levels of herbivore attack pressure (i.e. non-outbreak versus outbreak *M. janthinus* populations).

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Chapters 3 and 4 explore the third variable that contributes to predicting the success of a

biological control agent, the ecology of the control insect. One underlying objective of

biocontrol programs is to manipulate populations of natural enemies to induce outbreak

dynamics in order to reduce target weed densities (De Clerck-Floate and Bourchier,

2000). Crawley (1986a,b) found a correlation between the intrinsic rate of increase of

insect agents and the probability of their success. As Price (2000) described food as the

key resource that influences and modifies all other ecological relationships through the

trophic system, it was necessary to explore the bottom up (i.e. host plant) regulation of

M. janthinus within its new environment. Of particular interest was the examination of

effects of weevil population growth and resultant host impact on the population

characteristics of *M. janthinus* under reduced host availability. Chapter 3 evaluates the

relationships between the population density achieved by weevils from different

establishment periods and several insect quality estimates. Results of the study on

M. janthinus quality are discussed in terms of fitness, as per Roitberg *et al.* (2001). The

host selection behaviour of ovipositing *M. janthinus* and implications of these choices

under different levels of insect crowding and host availability on offspring performance

were explored in Chapter 4.

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TABLE 1.1. Summary of adult *M. janthinus* abundance counts across 17 study sites near Grand Forks, BC. Site types were categorized using the 2001 adult weevil counts as NO (non-outbreak) and OB (outbreak).

Site	Site Type	Release Year	Release Number	Adult Count (weevils/min)	
				2001	2002
ABH[abc]	OB	1998	400	38.7	35.1
Christina Lake[abc]	OB	1997	200	79.7	57.4
Gilpin 1.0[ac]	OB	1996	100	48.2	34.9
Gilpin 2.15[a]	OB	1997	200	61.3	36.1
Granby Dump[ac]	OB	1994	300	33.1	27.2
Lone Pine Pit[abc]	OB	1997	200	41.5	39.5
Morrissey[a]	OB	1994	300	46.6	18.7
Overton[ac]	OB	1994	300	27.4	38.0
Danshin[ac]	NO	2000	400	2.0	8.6
Eagle Ridge[ac]	NO	2000	400	6.8	33.9
East Gilpin[ac]	NO	1999	400	15.6	83.0
Norwegian[abc]	NO	2000	400	0	1.0
Ponderosa[b]	NO	2001	600	0.9	25.8
Rock Creek[ab]	NO	2000	400	1.3	0.4
Sand Ck. 0.5[ac]	NO	1998	400	13.6	58.0
Sank Ck. 2.0[ac]	NO	1999	400	6.1	27.1
Snowball[a]	NO	1999	600	4.0	3.3

[a] 30 permanent vegetation quadrats were established at these (n=16) sites and monitored in both 2001 and 2001 (Chapter 2)

[b] 40 plants were individually tagged from these (n=6) sites and monitored during the 2001 and 2002 field seasons (Chapter 2)

[c] 10 toadflax shoots were collected from these (n=12) sites and dissected in 2001 (Chapter 4)

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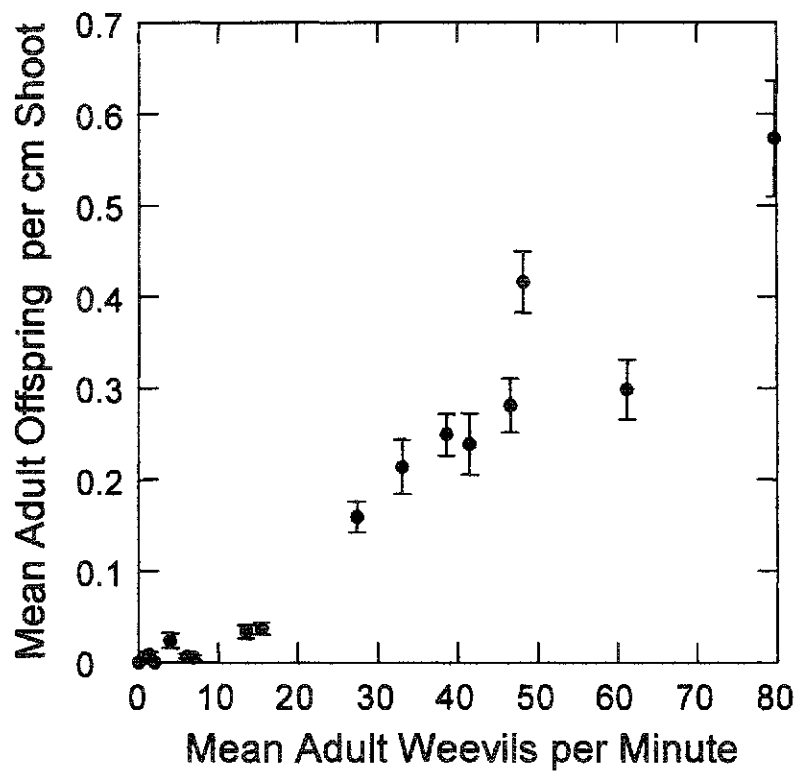
FIGURE 1.1. Relationship between 2001 *M. janthinus* adult counts and subsequent offspring densities within toadflax stalks across 16 study sites (excluding Ponderosa). Mean offspring densities were calculated from 30 randomly sampled, senesced toadflax stalks per site. r^2=0.934, P<0.001, y=0.007x, where y and x represent the density of adult offspring and parent adult weevil count, respectively.

CHAPTER 2

Impact of *M. janthinus* on Dalmatian toadflax

Introduction

Classical biological control of weeds can be regarded as "experimental plant population ecology" (Myers *et al.*, 1989). Many plants in alien environments experience increased vigour and successful establishment as a result of improved environmental conditions or escape from natural enemies (Blossey and Kamil, 1996; Crawley, 1986). Classical weed biocontrol relies on the use of herbivores to suppress target plant densities and to restrict seed production and dispersal (Isaacson *et al.*, 1996). By introducing biological control agents against invasive weeds, we also can measure the direct impact of herbivory on plant densities and other measures of plant performance, particularly within successful systems.

One of the biggest criticisms of classical biological control remains that post-establishment, field evaluation of the success of released agents is infrequent (Crawley, 1989; McClay, 1995). Although McEvoy (1985) recommends the use of manipulative field experiments to assess the impact of insect biocontrol agents, it is often not possible or practical to perform this type of study. One explanation for the lack of experimental design during the evaluation of introduced biological control agents is that agent establishment can be highly unpredictable (McEvoy, 1985). McFadyen (1998) also suggests that lack of financial sponsorship for post-establishment evaluation of

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introduced agents has been a problem in many biocontrol systems. Additionally,

establishing and sustaining insect-free "control" plots can be costly and present logistic

difficulties, especially when using highly mobile insect agents during long term studies

(McClay, 1995). Researchers often use observational studies to elucidate patterns of

herbivory and host plant dynamics (Fay *et al.*, 1996; Hoffman *et al.*, 1990). Although, in

many cases, results from surveys of natural populations can be difficult to interpret due to

the variability in host plant performance from other abiotic and biotic factors (Briese,

1996), the majority of impact studies examined by McClay (1995) employed the

observational approach (i.e. 72%).

Long term monitoring of initial *M. janthinus* Germar (Coleoptera: Curculionidae) release

sites in British Columbia (BC) showed a correspondence between increases in weevil

populations and the incidence and intensity of *M. janthinus* attack on Dalmatian toadflax,

Linaria dalmatica (L.) Mill., over a four-year period (De Clerck-Floate and Miller,

2002). Further evaluation was necessary to determine how observed levels of weevil

attack and insect population density influence reproductive and vegetative output of both

individual toadflax plants and populations of the weed. The current study evaluated the

relationships between *M. janthinus* attack and several life history traits of Dalmatian

toadflax (e.g. patterns of growth and reproduction, as in Begon *et al.*, 1990). Toadflax

plant growth and reproductive output were compared between recently established, low

weevil density sites (i.e. non-outbreak) and well-established, high density (i.e. outbreak)

sites. Previous studies (Jeanneret and Schroeder, 1992; Saner *et al.*, 1994) attributed host

impact by *M. janthinus* to larval feeding within toadflax shoots. It also was anticipated,

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prior to the release of *M. janthinus* in North America, that larval feeding would have the greatest impact (Jeanneret and Schroeder, 1991). Hence, novel to this study, the relationships between feeding by adult *M. janthinus* and above-ground vegetative growth and sexual reproduction of toadflax plants are specifically addressed. This study is observational in nature, examining the interactions between *M. janthinus* and its host plant under variable and ambient plant and insect densities post-weevil establishment.

Methods

Impact of *M. janthinus* attack on toadflax populations

Sixteen of the total 17 sample sites, varying in both time since weevil release and number of weevils originally released, were chosen in June 2001 from over 65 release sites in the Boundary district of the West Kootenay region. These represented a range of weevil establishment, based on adult weevil counts (Table 1.1). The sites were chosen to illustrate impact under different intensities of weevil attack and to evaluate the effects of early spring adult *M. janthinus* feeding on toadflax populations, particularly regarding reproductive potential. At each of these sites, thirty 0.25 m^2 permanent rectangular quadrats were established in a grid pattern (i.e. 5 rows of 6 quadrats, each row and quadrat spaced 2 m apart). Quadrats were located within a 20 m radius of the location of each original *M. janthinus* release. The mean proportions of shoots with apical meristem damage (i.e. shoot tip damage) caused by *M. janthinus* adult feeding, and the proportions of reproductive versus vegetative shoots were calculated per quadrat during 23-27 June,

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2001 and 18-21 June, 2002. Only shoot tips that had been destroyed by *M. janthinus* were counted as damaged. Feeding damage by adult weevils could be clearly distinguished from other causes of shoot injury. Shoots with visible flower buds or flowers were classified as reproductive; all other shoots were classified as vegetative.

Non-linear regressions (Bates and Watts, 1988) were performed on each season's data to evaluate the relationships between adult weevil abundance (see Chapter 1) and the mean proportions of shoot tips damaged and reproductive shoots by site. For these analyses, a general logistic curve was chosen based on initial scatter plot data:

$$y = \frac{e^{(ax+b)}}{1+e^{(ax+b)}}$$

where y represents either the mean proportion of shoot tips damaged per site or the mean proportion of reproductive shoots per site across the adult weevil abundance counts from the same sites (x). Both equation parameters (i.e. a and b) were iterated using Systat 10.2. The relationship between the mean proportions of shoot tips damaged by *M. janthinus* adults in each study season and the mean proportions of shoots that successfully developed flowers or flower buds by late-June was analyzed using linear regression. Data for the reproductive shoots were rank-transformed prior to the linear analysis, as data were not normally distributed (Conover and Iman, 1981). No data were transformed for analysis using the nonlinear regressions.

Impact of *M. janthinus* attack on individual toadflax plants

More intensive studies were made on selected plants at six study sites (Table 1.1) to determine the associations between *M. janthinus* abundance, adult and combined adult/larval attack damage and toadflax plant reproductive and vegetative production. At each study site, 20 plants with three to five robust shoots were selected within a 20 m radius of the point of original weevil release. Twenty plants of similar architecture and size were chosen at a 100 m distance from the release location. The ground around each plant was excavated to ensure that plants were discrete individuals prior to marking with flagging tape and a numbered metal tag. Shoot height, type (i.e. reproductive or vegetative) and the presence or absence of shoot tip damage caused by adult *M. janthinus* feeding, were recorded for each shoot of each plant during the weeks of 23 June, 2001 and 18 June, 2002. During the last weeks of August 2001 and 2002, the total numbers of shoots, and developed seed capsules per plant were recorded. Shoots from the tagged plants were collected in August of both years and a sub-sample of two randomly selected shoots per plant was dissected in the laboratory to determine the densities of offspring *M. janthinus* within shoots. These densities were used to estimate total developing weevil densities within each plant in each sample season. Comparisons of the proportions of damaged shoot tips and reproductive shoots per plant, sexual reproductive output and vegetative growth of toadflax plants were made across site categories (i.e. non-outbreak versus outbreak sites, n=3 sites per category) and sample years (2001 versus 2002) using GLM ANOVAs. The analysis of mean number of shoots produced by attacked plants also included sample period (i.e. late-June versus late-August) as a treatment factor. The data

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for each plant performance measurement were not normally distributed and, thus, rank transformed (Conover and Iman, 1981). Tukey HSD adjusted-pairwise comparisons were made across site types, sample years and sample periods where appropriate. Spearman correlation analyses were performed to evaluate the association between toadflax shoot tip damage caused by adult weevil feeding and above-ground reproductive and vegetative output of plants. Changes in seedpod yield, vegetative shoot production and linear shoot growth from 2001 to 2002 were correlated with *M. janthinus* offspring densities.

To estimate the impact of *M. janthinus* adult plus larval feeding on toadflax plant sexual reproduction, changes in seedpod number were translated into change in seed production based on mean seed counts per pod taken from unattacked toadflax plants in Ft. MacLeod in July 2002. One hundred seedpods were randomly collected from mature toadflax plants in a densely populated stand on three sample dates: 4, 15, 30 July. Sampling during the early seed production period (July-August) should reliably estimate the level of seed production, as Robocker (1970) found that ~97% of seeds were produced in the first five weeks of seed set. Sub-samples of 30 seedpods per collection date were dissected and total seed numbers assessed. A Kruskal-Wallis one-way ANOVA was performed to determine if the number of seeds per capsule were consistent across the reproductive season. Mean seed production was used to estimate the changes in reproductive output of toadflax plants in BC under different levels of weevil attack.

Data were analyzed using Systat 10.2 (linear regressions) and SPSS 10.0 (Spearman rank correlations). Unless otherwise stated, untransformed mean data are presented with accompanying standard errors.

Results

Impact of *M. janthinus* attack on toadflax populations

Levels of mean shoot tip damage at non-outbreak sites ranged from 0.1-53% in June 2001 (Table 2.1). By contrast, more established sites with outbreaking weevil populations had between 66% and 98% of all surveyed shoots with tip damage (Table 2.1). By June 2002, the mean proportions of shoot tips damaged at the same non-outbreak sites rose to 16-93% within quadrat samples (Table 2.1). No fewer than 80% of shoot tips were damaged in the quadrats surveyed at outbreak sites in 2002 (Table 2.1). Quadrat survey data from both 2001 and 2002 showed that there is a changing within-year relationship between the mean proportion of toadflax shoot tips damaged by adult *M. janthinus* feeding in June and the abundance of spring-emerged weevil populations (Fig. 2.1). At sites where adult weevil abundance was low in 2001 (i.e. at non-outbreak sites, 20 *M. janthinus* per minute count), the proportion of shoot tips damaged was below 40% (Fig. 2.1a). Above abundance counts of 40 weevils per minute, shoot tip damage levels were 90% or greater (Fig. 2.1a). Observed damage levels at non-outbreak sites were slightly higher in 2002 than 2001, with over 60% of shoot tips damaged as adult weevil counts reached 20 weevils per minute. As in the 2001 data, populations of *M. janthinus* in

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2002 reached levels of maximum host damage shortly after surpassing counts of 40 adult weevils per minute (Fig. 2.1b).

The damage caused by intensive adult *M. janthinus* feeding on toadflax apical meristems resulted in significant reductions in the mean proportions of reproductive shoots within surveyed toadflax stands (Table 2.2). Much lower proportions of shoots that would become reproductive were observed at sites with high levels of adult feeding damage (i.e. outbreak) (Fig. 2.2a,b). Non-outbreak sites had, in general, from 3-30 times more reproductive shoots within survey quadrats in 2001,and 5-47 times more reproductive shoots in 2002 than in quadrats from outbreak sites. The mean proportion of reproductive shoots per quadrat was also a function of adult weevil abundance at study sites, with an effectively complete loss of reproductive shoots in both 2001 and 2002 as *M. janthinus* counts reached about 50 weevils per minute in both sample years (Fig. 2.2c,d). Above this weevil abundance level, shoots with flowers and/or buds represented only 1-4% of the total shoot population within quadrats)(Fig. 2.2c,d).

Impact of *M. janthinus* attack on individual toadflax plants

Separating toadflax plant attack data by the proximity of plants to the original *M. janthinus* release area (i.e. within a 20 m radius of the release point versus 100 m away), or by individual study site, did not improve the fit of any of the ANOVA models, thus tagged plants were pooled across site categories for analysis (n=120 plants per category). Shoot tip damage per plant was not significantly different between the sample

years ($F_{1,474}$=1.518, P=0.219) in the ANOVA model (r^2=0.344, n=478 plants). In both

sample years, there was a distinct pattern of increased shoot tip damage to plants at sites

with high adult weevil counts (Fig. 2.3a). Plants at outbreak sites had over 45% of their

shoots attacked in June 2001 (68%) and 2002 (47%), whereas shoot tip damage per plant

was 5% (2001) to 20% (2002) at sites with newly established weevils ($F_{1,474}$=198.292,

P<0.001). The interaction between sample year and site category was significant

($F_{1,474}$=47.522, P<0.001), likely due to an unexpectedly large annual *M. janthinus*

population increase (25-fold) in one of the three non-outbreak sites used in the study

(Ponderosa, 2001 release).

Adult *M. janthinus* feeding on apical shoot tissues in early spring 2001 and 2002 was

related to reductions in the proportion of shoots per plant that successfully became

reproductive (Fig. 2.3b, Table 2.2). From the ANOVA model (r^2=0.344, n=473 plants), it

was determined that plants from outbreak sites had significantly fewer reproductive

shoots than those at non-outbreak sites ($F_{1,469}$=193.531, P<0.001). Reproductive shoots

represented only 17% and 8% of the total shoots on plants at outbreak sites in 2001 and

2002 respectively, whereas 39-67% of the shoots on non-outbreak plants had flowers or

flower buds when sampled in late June (Fig. 2.3b). There were significant differences

between the proportions of reproductive shoots on plants in 2001 and 2002

($F_{1,469}$=43.323, P<0.001), with 9-29% fewer shoots per plant becoming reproductive in

2002. A significant interaction between site category and sample year was also present

($F_{1,469}$=9.717, P=0.002).

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Attack by *M. janthinus* adults on apical meristem tissues was highly associated with reduced seedpod production in both 2001 and 2002 (Table 2.2). Higher adult weevil counts corresponded to reduced variability in seedpod output from 2001-2002 (Fig. 2.4). Heavily attacked plants at outbreak sites produced significantly fewer seedpods in both 2001 and 2002, as compared to plants at non-outbreak sites ($F_{1,394}=321.939$, $P<0.001$). Plants at non-outbreak sites produced over twice as many seedpods as plants at outbreak sites in 2001 and 2002 (Fig. 2.5a). Seedpod production was significantly higher in 2001 ($F_{1,394}=62.642$, $P<0.001$), according to the ANOVA model ($r^2=0.492$, n=398 plants). There was no significant interaction between plants from different site categories and sample years ($F_{1,394}=0.512$, $P=0.475$). The pooled mean number of seeds ($\mu=328\pm6$ seeds, n=90 pods) from seedpod collections at Ft. MacLeod, Alberta was used to estimate the sexual reproductive output of toadflax plants in BC (n=30 seedpods/sample date, Kruskal-Wallis test statistic for sample date=1.605, df=2, $P=0.448$). By this estimate, individuals from non-outbreak sites would produce 73,024-96,822 seeds, whereas plants from outbreak sites achieved a production of only 25,428-45,314 seeds.

Adult *M. janthinus* feeding damage also had a strong negative correlation with the total linear length of shoot material produced by toadflax plants during the June sample period (Table 2.2). Plants at weevil outbreak sites were, on average, 1.5-4 times shorter than those at non-outbreak sites ($F_{1,476}=168.039$, $P<0.001$), according to the ANOVA model ($r^2=0.409$, n=480 plants) (Fig. 2.5b). The size of plants, as measured by total linear length, was significantly reduced in 2002, as compared to the 2001 sample

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($F_{1,476}$=150.961, P<0.001) and the interaction between sample year and site category was significant ($F_{1,476}$=9.854, P=0.002).

The proportions of shoots with adult *M. janthinus* feeding damage were negatively associated with the total numbers of shoots produced by the sampled plants in August 2002 (Table 2.2). Plants were initially chosen under the criteria of three to five robust upright shoots in June 2001. Counts of shoots per plant were thus similar in June and August 2001 at both outbreak and non-outbreak sites (Fig. 2.6; Table 2.2). The full ANOVA model (r^2=0.132, n=861 plants) showed three significant interactions; site category versus sample year ($F_{1,853}$=24.426, P<0.001), site category versus sample period (i.e. June or August) ($F_{1,853}$=4.060, P=0.044), and sample year versus sample period ($F_{1,853}$=22.689, P<0.001). The full model was subsequently reduced by sample period. Analysis of plants surveyed in June (r^2=0.171, n=463 plants) showed that site category ($F_{1,459}$=43.226, P<0.001), sample year ($F_{1,459}$=33.064, P<0.001) and the interaction between the two factors ($F_{1,459}$=22.951, P<0.001) had significant effects on the number of shoots produced. This resulted from reduced shoot recruitment in June 2002 in plants under heavy attack (i.e. at outbreak sites) (Fig. 2.6a). Plants surveyed in August (r^2=0.043, n=398 plants) had significantly different numbers of shoots by site category ($F_{1,394}$=10.456, P<0.001). Patterns of mid-summer shoot recruitment were consistent across sample years ($F_{1,394}$=1.524, P=0.218). The interaction between site category and sample year resulted in significant differences in the numbers of shoots per plant during the August sample period ($F_{1,394}$=5.506, P=0.019). There was significantly increased shoot recruitment in August 2002 at non-outbreak sites (Fig. 2.6b).

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The previous year's *M. janthinus* offspring densities (2001) within plants showed

interesting associations with the annual change in plant production parameters measured

above (Table 2.2). Similar to the patterns of adult weevil impact on seedpod production,

higher larval densities within shoots, representative of the combined adult and larval

attack in 2001, were associated with a decreased seedpod production from 2001-2002. At

the maximum offspring density of 302 weevils per plant in 2001, one sample plant

produced 553 less seedpods, or 181,384 fewer seeds, in 2002 than it did in 2001. Plants

with minimal insect attack the previous year (i.e. plants at non-outbreak sites) also

suffered a reduced mean seedpod production of 86±28 pods in 2002, translating into

19,024-37,392 fewer seeds. *M. janthinus* larval infestation rates in 2001 were associated

with increased shoot production at the individual plant level from 2001-2002, as

measured by both total linear shoot length and number of shoots produced (Table 2.2).

Discussion

Adult *M. janthinus* feeding damage makes a direct and substantial contribution to

reducing the levels of reproduction and plant vigour of the target host, *L. dalmatica*. This

was unexpected (A. Gassmann, pers. comm.), as previous studies of this biocontrol

agent's impact indicated that *M. janthinus* larvae were primarily responsible for reducing

toadflax shoot biomass (Saner *et al.*, 1994), seed production and inducing premature

shoot wilting (Jeanneret and Schroeder, 1991). In Spokane, Washington, seedling

emergence is in late-March (Robocker, 1970). In a similar climate in BC, adult *M.*

janthinus emerge from the previous years' toadflax stalks during April-May (De Clerck-Floate and Miller, 2002) and begin to feed on young, succulent toadflax shoots prior to shoot differentiation. Early-season adult herbivory on shoot meristem tissues renders toadflax shoots that may have been predetermined to become reproductive "functionally asexual" (see Taiz and Zeiger, 1991) and causes shoot stunting, similar to the effects of herbivory by the flower beetle, *Brachypterolus pulicarius*, on Dalmatian toadflax (Grubb et al., 2002). The results of adult *M. janthinus* feeding include reduced seedpod production, above-ground biomass production of individual toadflax plants and, consequently, dramatically decreased reproductive potential of toadflax populations.

Timed counts of *M. janthinus* adults, as an estimation of population abundance, proved to be highly predictive of the levels of attack and impact on toadflax populations. Despite the chosen study sites having variable original release numbers and years, strong patterns emerged from the data. Both study years showed that as *M. janthinus* populations reached counts of 40 weevils per minute, nearly 100% damage to shoot tips occurred (Fig. 2.1). Damage levels were recorded to have risen 16-40% from spring 2001-2002. Resulting from this apical shoot damage was suppression in the production of flower buds and flowers (Figs. 2.2a,b), with a pattern of *M. janthinus* abundances over 50 weevils per minute virtually eliminating all of the reproductive shoots in the studied toadflax populations (Fig. 2.2c,d). Patterns of reproductive shoot production within a single sample season (2001) decreased from over 20% under low weevil attack (i.e. non-outbreak sites) to less than 1% under intense stress from adult weevil feeding (i.e. outbreak sites) (Fig. 2.2a). These observations indicate that *M. janthinus* populations that

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establish and achieve abundance levels of at least 40-50 weevils per minute have the potential to directly reduce the sexual reproductive success of *L. dalmatica* stands 20-fold generally within 3-4 years of release (see Fig. 3.1).

The patterns of decreased reproductive potential and vigour of toadflax populations under intense *M. janthinus* herbivory were confirmed and explained at the individual plant level. The intent of monitoring discrete toadflax plants over two growing seasons was to elucidate the mechanisms by which *M. janthinus* impacts its host in order to understand the reductions in toadflax shoot density that have been observed (R. A. De Clerck-Floate, unpublished, in De Clerck-Floate and Harris, 2002). Between 47% and 68% of shoot tips per plant were attacked by adult *M. janthinus* under outbreak weevil populations in June of both sample years, whereas most of the plants surveyed at sites with non-outbreaking levels of weevil abundance had well below 20% apical meristem damage (Fig. 2.3a). Similarly, a strong association in 2001 between adult feeding damage and the number of reproductive shoots present in the June survey was found. Figure 2.3b shows 3-4 times more shoots with flowers or flower buds at non-outbreak study sites than those under intense herbivory pressure from outbreaking adult weevil populations. The growth of flowering shoots in *L. dalmatica* occurs in one spring flush (Saner *et al.*, 1994), coincident with the emergence and feeding of adult weevils in BC (De Clerck-Floate and Miller, 2002). Robocker et al. (1972) suggested that, since toadflax plants senesce when top growth is removed, shoots do not have the capacity to regenerate photosynthetic tissue. Consequently, toadflax plants do not have the ability to compensate for adult

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weevil feeding later in the growing season through the growth and dedication of new reproductive shoots (Saner *et al.*, 1994).

A significant reduction in the number of developed seedpods was observed in heavily attacked plants compared to those at non-outbreak sites (Fig. 2.5a). Seedpod production from plants experiencing intense feeding pressure by adult weevils (i.e. plants at outbreak sites) was less than half of that from plants with low adult *M. janthinus* abundance (i.e. plants at non-outbreak sites). Additionally, the variability in seedpod production from one sample season to the next is highly reduced as adult weevil herbivory increases (Fig. 2.4). These findings, in conjunction with highly negative associations between apical shoot damage and seedpod production in 2001 (r_s=-0.702, *p<0.001*) and 2002 (r_s=-0.750, *p<0.001*) suggest that adult feeding damage may be a key regulating factor in toadflax fitness in BC.

The estimates of overall seed production of the BC study plants were extrapolated from the mean number of seeds produced (μ=328±6 seeds/pod) by unattacked *L. dalmatica* plants from Ft. MacLeod, Alberta during July 2002. These observations are slightly higher than the seed production estimates made by Robocker (1970), but should reliably approximate the baseline reproductive potential of seedpods in healthy plants, unstressed by weevil herbivory. Potential site-specific differences in seed numbers notwithstanding, plants produced 50-66% fewer seeds at outbreak sites in BC using the above estimate of production. It has yet to be determined, however, whether the number or viability of seeds is reduced under high levels of herbivory. Root-mining in yellow toadflax (*Linaria*

vulgaris (L.) Mill.) has been proven to shorten the flowering season and lower seed weights (Saner *et al.*, 1994). This type of effect on the vigour of Dalmatian toadflax by *M. janthinus* stem-boring needs to be examined to fully understand the impact of this weevil on its host.

Another effect of adult *M. janthinus* feeding on apical shoot growth was the occurrence of shoot stunting. The total linear lengths of study plants in June were highly, negatively associated with adult feeding damage in both 2001 (n=240, r_s=-0.518, $p<0.001$) and 2002 (n=158, r_s=-0.586, $p<0.001$). Plants experiencing high levels of adult *M. janthinus* attack had 1.5-4 times shorter shoots than those with low levels of adult weevil herbivory. The stunting that resulted from meristem damage appears to have prevented plants from attaining the level of above-ground biomass an unattacked plant would otherwise achieve. This corresponds to the controlled study by Saner *et al.* (1994), indicating that combined adult and larval feeding and oviposition reduced shoot biomass of Dalmatian toadflax significantly. Adult *M. janthinus* feeding, consequently, directly alters toadflax plant architecture in addition to reducing sexual reproductive potential. This also translates into reduced food for subsequent generations of attacking *M. janthinus*.

It has been previously documented that stem-boring by multiple larvae within a shoot causes premature wilting of shoots and contributes to a suppression in flower formation (Jeanneret and Schroeder, 1992) and there have been predictions that a shoot-borer would reduce the competitive abilities of attacked shoots (Jeanneret and Schroeder, 1991). The current study could not measure the isolated contribution of *M. janthinus* larvae in

reducing reproductive and vegetative growth. It was possible, however, to observe the effects of combined adult and larval feeding on these plant performance parameters. Utilizing the density of offspring within toadflax shoots in 2001, as a measurement for total insect attack that year, this study showed that the annual production of seedpods was reduced in association with higher rates of overall attack (r_s=-0.272, P=0.021). Contrary to the predictions of this study and the findings of Saner *et al.* (1994), shoot mining was somewhat associated with an increased above-ground biomass production at the individual plant level (r_s=0.247, P=0.038) and strongly associated with an increased number of shoots per plant produced between 2001-2002 (r_s=0.476, P<0.001). Figure 2.6 provides a potential explanation for these results. It appears that under intense weevil herbivory (i.e. outbreak *M. janthinus* populations) during the previous growing season, recruitment of toadflax shoots was suppressed in June 2002. This trend, however, was reversed during the remainder of the growing season after adult weevil feeding ceased, resulting in a slight, but not significant, increase in the number of secondary shoots produced by the end of August. The vegetation produced during this period consisted of small, weak offshoots, many of which died prior to sampling. Vujnovic and Wein (1997) document a similar response in clipping experiments of toadflax primary shoots in June, showing the production of small axillary stems from dormant buds. Under low weevil attack rates (Fig. 2.6, non-outbreak) in 2002, the number of secondary shoots produced by plants in mid to late-summer increased substantially. The patterns of shoot recruitment in *L. dalmatica* plants indicate that there may be a weak, or under-compensatory, response to *M. janthinus* damage. Few papers have demonstrated over-compensation by plants in response to herbivory, as in McNaughton (1983).

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These patterns of shoot production suggest that the repeated and intensive feeding by both adult and larval *M. janthinus* reduces toadflax vigour, as a result of decreased quality of its above-ground biomass. Mid-season growth of prostrate stems (i.e. those arising from adventitious stem buds off vertical or lateral roots; Robocker, 1974) is known to contribute to the energy reserves stored in the roots of a toadflax plant (Saner *et al.*, 1994), and the development of floral stems the following year is most often associated with the carbohydrate reserves contributed by these prostrate stems (Robocker, 1974). Intense weevil feeding pressure in the previous season reduced the vigour of toadflax plants and prevented them from producing as many potential floral stems in 2002 as they could otherwise (Fig. 2.5b; Fig. 2.6). A similar result has been shown in shoot clipping experiments, where combined fall and subsequent spring clipping of plants reduced both carbohydrate reserves in roots and flowering in the second year (Robocker *et al.*, 1972). A weak growth response by toadflax plants in the mid-season production of many small prostrate stems is not enough to compensate for the direct damage caused by *M. janthinus* feeding. There is little evidence that herbivory actually benefits plants outside of crop systems (Belsky, 1986).

The weevil *M. janthinus* is a highly effective biological control agent for the suppression and reduction of its target weed, Dalmatian toadflax. Direct effects of *M. janthinus* feeding on *L. dalmatica* have been quantified within this study, as well as by other researchers (Jeanneret and Schroeder, 1991, 1992; Saner *et al.*, 1994). According to Maschinski and Whitham (1989), the impact of herbivory largely depends on the amount

and type of plant tissues removed, combined with the timing of attack and its relation to the life cycle of the host plant. The success of *M. janthinus* as a biological control agent can be largely attributed to these attack mechanisms:

1) Early season adult weevil feeding results in serious reductions in flowering and seed production potential of toadflax plants. As the survival of toadflax stands relies on floral and subsequent seed production (Robocker, 1974), *M. janthinus* targets the most important mechanism for *L. dalmatica* persistence in its environment.

2) High levels of combined adult and larval feeding reduce the above-ground vegetative production of toadflax plants by stunting shoots during their primary growth phase. According to Robocker *et al.* (1972), carbohydrate reserves are at their lowest levels during peak plant growth and, as a consequence, removal of top-growth during this period produces the least amount of plant regrowth. This, combined with the construction of larval mines that disrupts the transfer of phytosynthetic products to root tissues, allow *M. janthinus* acts as an energy sink (Saner *et al.*, 1994).

There are also important indirect consequences of *M. janthinus* feeding that have not been quantified during this study but merit further evaluation. Saner *et al.* (1994) has already indicated that oviposition and stem-boring by *M. janthinus* causes structural damage to shoots, inducing water stress and as a result, increasing shoot mortality. Apical shoot damage by another toadflax natural enemy, *Brachypterolus pulicarius*, stimulates branching of toadflax shoots and diverts plant energy reserves from flowering and seed

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production (Grubb *et al.*, 2002). A similar consequence resulting from *M. janthinus* adult feeding should be explored. Seed quality and seedling establishment should be evaluated in toadflax populations experiencing intense attack and energy reserve depletion, as reductions in these plant performance indicators are well-documented consequences of herbivory (Bentley *et al.*, 1980; Louda, 1982; Parker and Salzman, 1985). Jeanneret and Schroeder (1991) speculated that *M. janthinus* feeding on *L. dalmatica* should greatly reduce the aggressiveness of this weed. This study concurs that toadflax vigour is reduced under intense herbivory. Questions regarding how the different levels of *M. janthinus* herbivory influence the competitive potential of toadflax within a plant community framework remain unanswered. Studies on post-weevil release plant community succession would address this hypothesis. Finally, and potentially most importantly, one long term effect of *M. janthinus* herbivory on Dalmatian toadflax is its potential to reduce the ability of *L. dalmatica* to respond to changing environmental conditions through reduction in seed production. Fewer seeds are available to provide the foundation for selection of more herbivore-tolerant phenotypes, drought resistance, high growth rates and other adaptive characteristics (Grubb *et al.* 2002).

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TABLE 2.1. Summary of the mean proportions of shoots damaged by spring adult *M. janthinus* feeding in 2001 and 2002. Data are presented as mean shoots damaged per 30 quadrats (±SE) at each study site (n=16 sites), designated as non-outbreak (NO) or outbreak (OB).

Site Name	Site Type	% Shoot Tips Damaged	
		2001	2002
ABH	OB	98.3 (1.2)	96.7 (2.2)
Christina Lake	OB	95.6 (3.4)	93.3 (6.7)
Gilpin 1.0	OB	92.5 (3.3)	81.0 (10.3)
Gilpin 2.15	OB	91.3 (3.7)	93.9 (3.4)
Granby Dump	OB	76.6 (4.7)	94.1 (2.0)
Lone Pine Pit	OB	95.5 (2.8)	87.8 (6.0)
Morrissey	OB	72.9 (7.8)	83.9 (5.9)
Overton	OB	66.0 (5.8)	96.5 (4.6)
Danshin	NO	10.7 (4.2)	35.9 (5.7)
Eagle Ridge	NO	19.3 (4.8)	90.9 (3.2)
East Gilpin	NO	53.2 (5.9)	93.0 (5.1)
Norwegian	NO	0.1 (0.1)	16.7 (5.1)
Rock Creek	NO	25.2 (4.5)	33.8 (5.6)
Sand Ck. 0.5	NO	36.9 (4.8)	88.2 (5.3)
Sand Ck. 2.0	NO	12.4 (5.4)	67.6 (4.8)
Snowball	NO	11.9 (5.0)	42.5 (7.8)

TABLE 2.2. Summary of Spearman correlations between *M. janthinus* attack measurements and toadflax growth and reproduction parameters per plant at all study sites. Measurements of plant parameters were either made in June (Jun) or August (Aug).

Attack Measurement	Plant Parameter	Sample Year	n	r_s	P
% shoot tips damaged	# Reproductive shoots/plant (Jun)	2001	240	-0.586	<0.001
		2002	231	-0.107	<0.001
% shoot tips damaged	# Seedpods/plant (Aug)	2001	240	-0.702	<0.001
		2002	158	-0.750	<0.001
% shoot tips damaged	Total linear length of plant (Jun)	2001	240	-0.518	<0.001
		2002	158	-0.586	<0.001
% shoot tips damaged	# Shoots/plant (Aug)	2001	240	-0.068	0.297
		2002	158	-0.185	0.020
2001 offspring density	Seedpod change 2001-02		72	-0.272	0.021
	Linear length 2001-02		71	0.247	0.038
	Shoot numbers 2001-02		72	0.476	<0.001
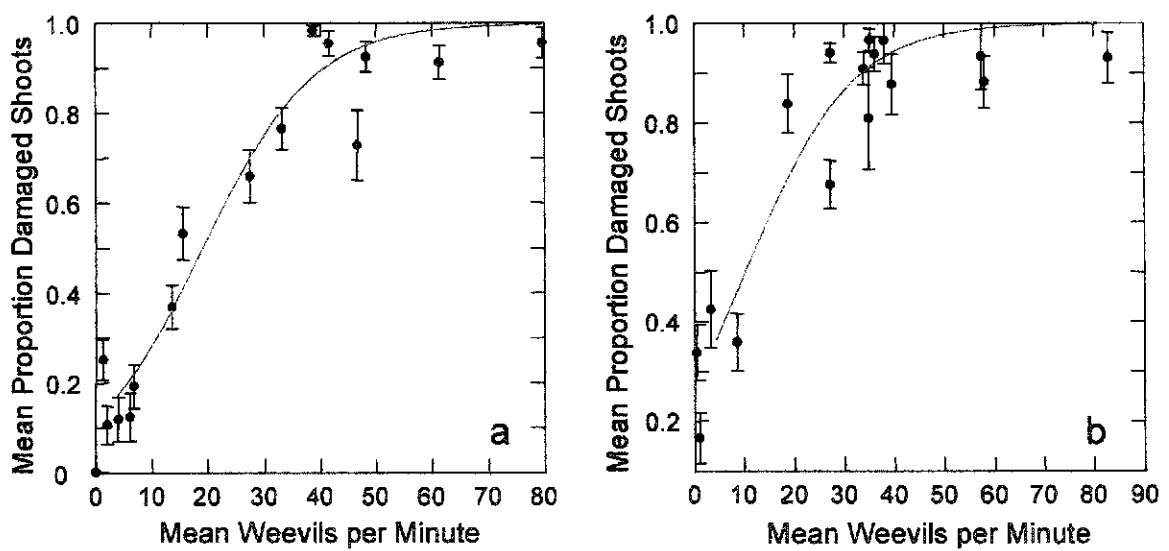
FIGURE 2.1. Toadflax shoot tip damage as a function of the abundance of adult *M. janthinus* at study sites. Means ± standard errors are presented for each study site (n=16 sites, 30 quadrats per site). Results include: (a) 2001, $y=e^{(0.103x-2.014)}/(1+e^{(0.103x-2.014)})$, $r^2=0.941$, and (b) 2002, $y=e^{(0.095x-0.982)}/(1+e^{(0.095x-0.982)})$, $r^2=0.883$, where y and x represent the mean proportion of shoot tips damaged and mean number of adult weevils per minute count, respectively.
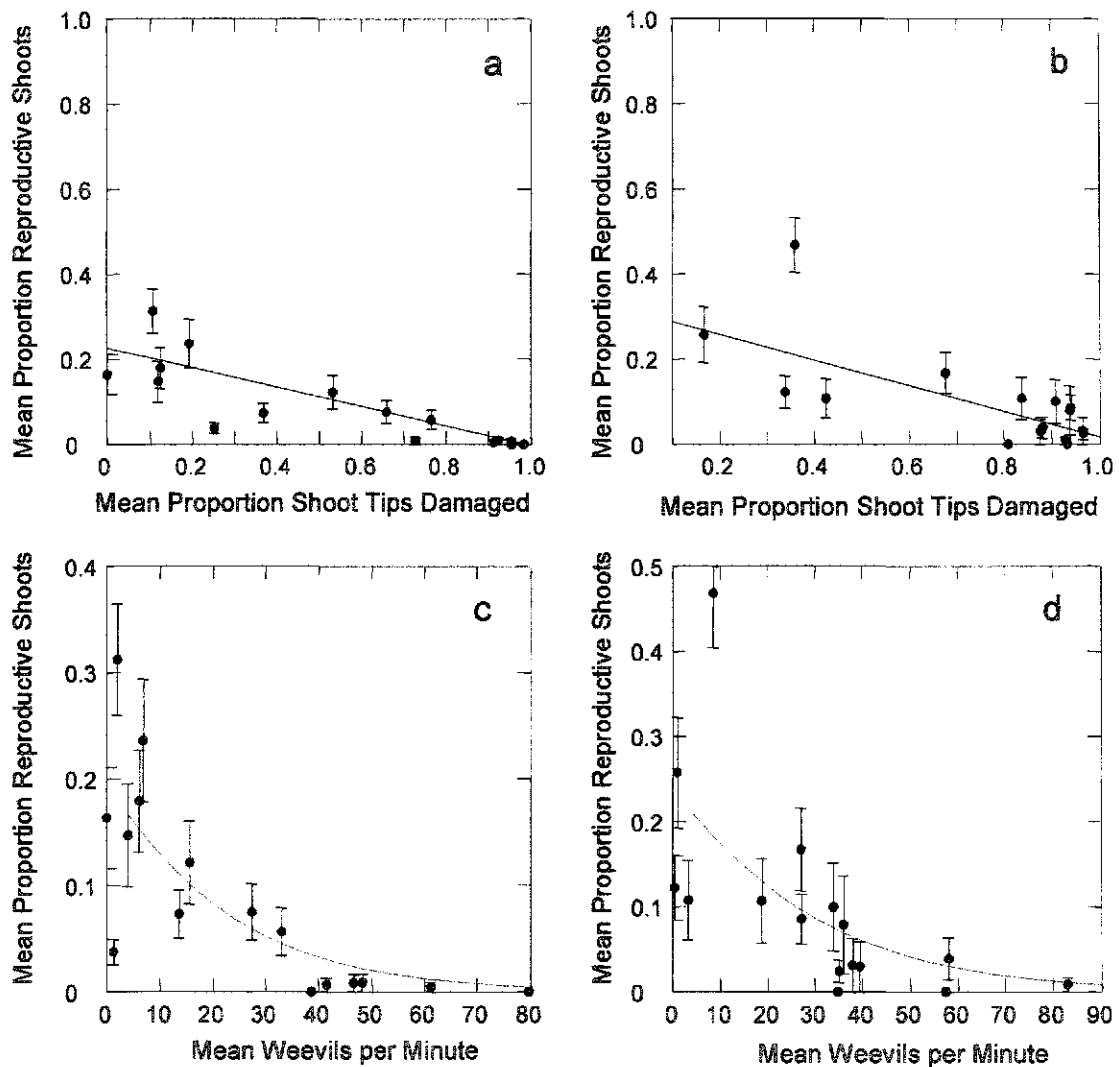
FIGURE 2.2. Incidence of reproductive toadflax shoots in late-June as a function of the proportions of shoot tips damaged and adult weevil abundances. Means ± standard errors are presented for each study site (n=16). The proportion of shoots that are reproductive (y) are evaluated in (a) 2001, y=0.206-0.217x, r^2=0.681, P<0.001, (b) 2002, y=0.344-0.324x, r^2=0.526, P<0.001, where x = mean number of shoot tips damaged, and (c) 2001 adult weevil abundances, y= $e^{(-0.050x-1.406)}$/(1+$e^{(-0.050x-1.406)}$), r^2=0.610, and (d) 2002 adult weevil abundances, y= $e^{(-0.041x-1.151)}$/(1+$e^{(-0.041x-1.151)}$), r^2=0.422, where x = the number of adult weevils per minute count.

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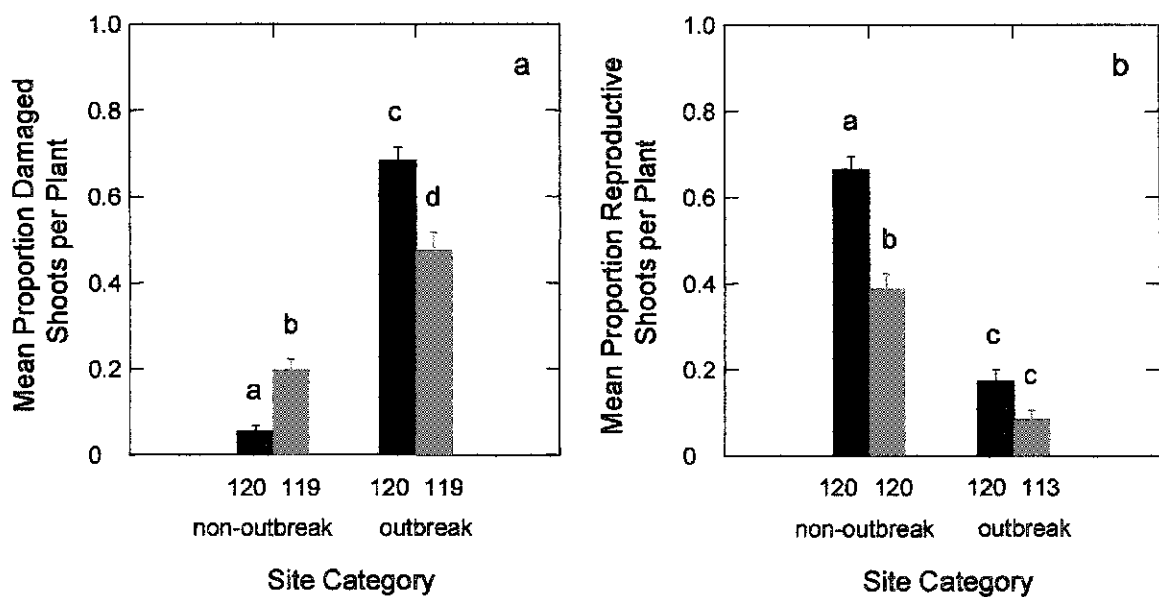
FIGURE 2.3. Comparisons of (a) levels of shoot tip damage from adult weevil feeding, and (b) proportions of reproductive shoots, across weevil abundance categories (non-outbreak vs. outbreak). Means ± standard errors are presented for plants sampled in 2001 (■) and 2002 (▨). Numbers of plants sampled in each group are presented below the x-axis. Different letters above bars represent means that are significantly different with 95% confidence.
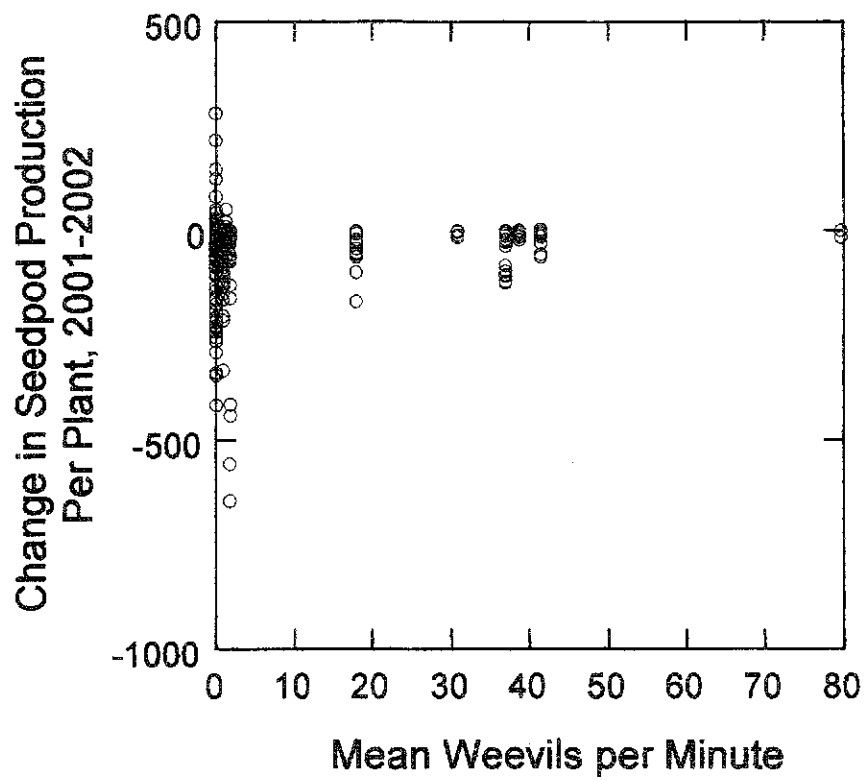
FIGURE 2.4. Reduction in the variation in toadflax seedpod production as adult weevil densities increase. The difference in total number of seedpods produced by each plant from 2001-2002 is presented (n=240 plants selected) versus adult *M. janthinus* abundance counts.
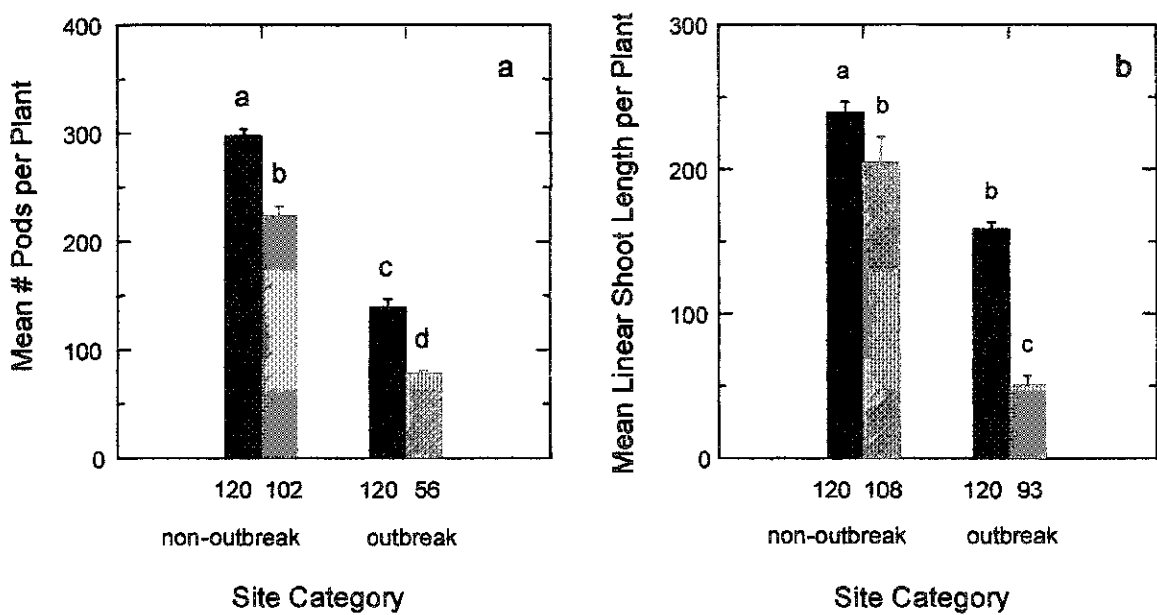
FIGURE 2.5. Differences in (a) toadflax seedpod production in August plant samples, and (b) total linear length of toadflax shoots per plant in June samples, across *M. janthinus* abundance categories (non-outbreak vs. outbreak). Means ± standard errors are presented for 2001 (■) and 2002 (▨). Numbers of plants sampled in each group are presented below the x-axis. Different letters above bars represent means that are significantly different with 95% confidence.

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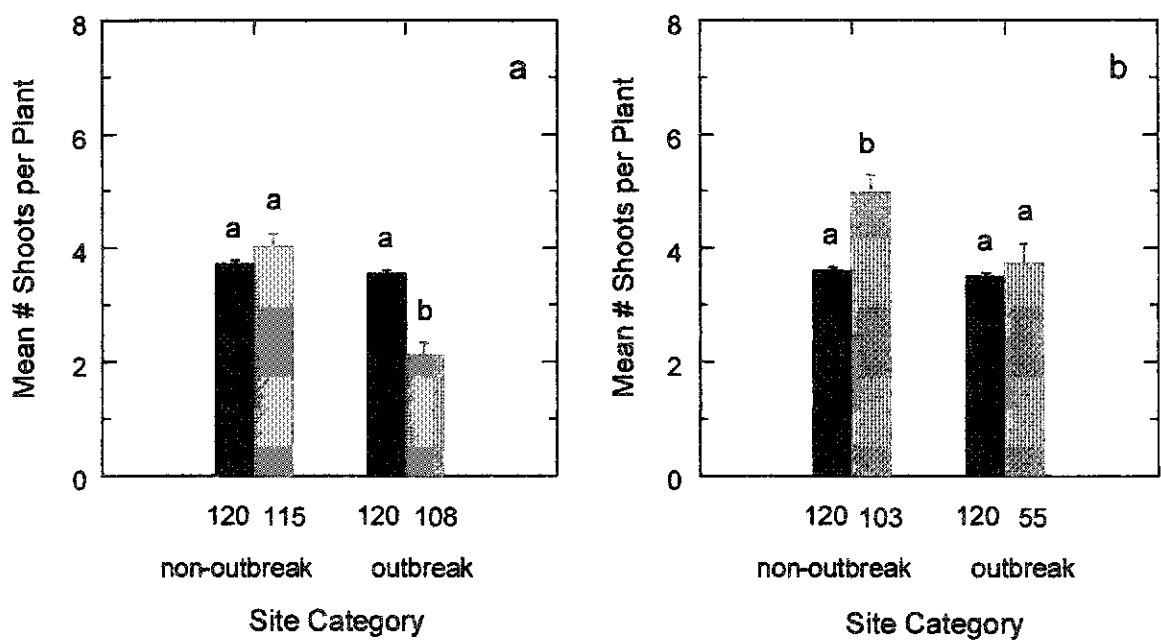
FIGURE 2.6. Comparison of toadflax shoots production by plants across *M. janthinus* abundance categories (outbreak vs. non-outbreak) in (a) June and (b) August. Means ± standard errors are presented for 2001 (■) and 2002 (▓). Numbers of plants sampled in each group are presented below the x-axis. Different letters above bars represent means that are significantly different with 95% confidence.

CHAPTER 3

Density-related changes in outbreaking *M. janthinus* populations: an evaluation of insect attributes relating to fitness

Introduction

Outbreak (i.e. eruptive) insect herbivore populations, as defined by Price *et al.* (1990), are expected to follow a cyclic trajectory from expansion to decline as a result of density-influenced negative feedback (Berryman, 1987). The "epidemic" phase of eruptive herbivorous insect population cycles is generally characterized by high insect densities that cause heavy damage to host species (Eber *et al.*, 2001). Endophagous specialist herbivores are highly dependent on their host plant resources (Roininen *et al.*, 1996) and would be likely candidates for strong regulation by bottom-up (i.e. host plant) forces resulting from negative density-dependence (Ylioja *et al.*, 1999).

Detailed studies of outbreak herbivore dynamics are generally limited to crop (Port and Guile, 1987) and forest pest species (Barbosa and Schultz, 1987; Hunter, 1991; Price *et al.*, 1990), but another area of entomology that is beginning to realize a need for in-depth study of insect population dynamics is classical weed biocontrol. Research in this field has traditionally included pre-release host specificity testing, the development of release strategies and determining factors affecting establishment (McEvoy and Coombs, 1999; McFadyen, 1998). However, the practice of classical biological control attempts to release insects "in such a manner that an agent outbreak is produced, which will reduce

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the density of the targeted weed to an acceptable threshold level" (De Clerck-Floate and Bourchier, 2000). Successful development of population dynamics models for introduced weed biocontrol agents (McEvoy and Coombs, 1999) could potentially result in substantial improvements of impact on the target host, establishment of biocontrol agents and the better predictability of agent performance (De Clerck-Floate and Bourchier, 2000).

M. janthinus, has been established for at least 8 years in some areas of the Southern interior of British Columbia (BC) and has achieved extremely high densities at some sites. Consequently, this weevil is being harvested and widely redistributed from these established 'nursery sites' (Harley and Forno, 1992) to new toadflax patches throughout BC. The implications of such rapid population growth on the vigour of individual weevils has not been previously ascertained and may have a bearing on the quality of insect populations being collected from nursery sites and used for new releases made in the province. Although there have been some studies evaluating the economic feasibility and viability of rearing weed biocontrol insects in captive or laboratory settings for augmentative release (Parrella and Kok, 1979; Story *et al.*, 1996; Stoyer and Kok, 1986), no studies to date have evaluated the quality of weed biocontrol agents produced by field nursery sites.

The current study provides a launching point from which a model of the outbreak population dynamics of the biological control agent, *M. janthinus*, can eventually be produced. This is not a thorough examination of the factors regulating this endophagous

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herbivore, as may be achieved by collecting detailed life-table data (Stiling, 1988). There

was, however, an unique opportunity with this weed biocontrol agent to look at the

influences on and population attributes of insects that establish and reach outbreak levels

within a comparatively short time period. A body size comparison of adult *M. janthinus*

was made across time since release to determine whether factors such as larval crowding

and resource decline affected insect vigour in a density-dependent manner. The term

'vigour' is used in this paper to describe the physiological and morphological condition

of individuals within a population (i.e. primarily adult size) that may influence their

tolerance to environmental factors (Sahota and Thomson, 1979). To determine whether

the size variation within a population of *M. janthinus* translated into changes in fitness (as

defined by Roitberg *et al.*, 2001), the association between adult female *M. janthinus* size

and potential fecundity was measured. Understanding the population dynamics and

attributes of established biological control insects prior to redistribution from high-

density nursery sites may contribute substantially to the improved application of these

agents for successful weed biocontrol.

Methods

Study populations

Individual adult *M. janthinus*, along with stalks of Dalmatian toadflax containing mature

weevils, were collected from established populations within or near Grand Forks, BC and

used in this study to determine whether the quality of individual *M. janthinus* declines

post-establishment, as populations of weevils become increasingly crowded and experience reduced host availability. Assessments of adult weevil abundance were made at 17 study sites in the Grand Forks, BC area (Table 1.1) between 25-29 June, 2001, and again between 18-22 June, 2002. These abundance measures were made using an observational count technique developed by R. A. De Clerck-Floate (unpublished) (see Chapter 1). Two observers each made a single count per study site in 2001, and two counts apiece in 2002. The mean number of *M. janthinus* adults counted per minute was determined on a per site and sample year basis. Adult weevil abundance levels were compared across the number of years since the original release and sample year using a two-way ANOVA to determine if population levels followed a pattern of establishment and population growth. Count data were square-root transformed prior to analysis in order to improve model fit (Zar, 1999). Populations of *M. janthinus* were designated as having reached outbreak levels when abundance counts exceeded 20 weevils per minute, as defined in Chapter 1.

Size variation and sex ratios of field-collected *M. janthinus* adults

Populations with adequate numbers of *M. janthinus* adults for collection (14 of the total 17 study sites) were randomly sampled in the Grand Forks, BC area during 19-25 June, 2002 by hand collection of approximately 150 adult weevils per site from Dalmatian toadflax stems and foliage. Sampled populations varied in age (i.e. years post-release), with different numbers of sites sampled per release age (n): 1 year post-release (n=1), 2 years (n=2), 3 years (n=2), 4 years (n=2), 5 years (n=3), 6 years (n=1), and 8 years (n=3).

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The numbers of sites sampled per release age were low and variable due to the limitation on availability of suitable study sites from any given year. For example, the BC Ministry of Forests made numerous *M. janthinus* releases in 1994, but very few in 1995 and 1996. Thus, sample sites were initially selected for the overall study based on adult weevil field counts, rather than release year.

Collected weevils were stored in glass vials containing 70% ethanol plus 5% acetic acid. Fifty randomly selected weevils were removed from each preserved sample and air dried on paper towels for 1-2 hours prior to measurement. Sex determination and body measurements were performed using a Leica MZ9.5 stereoscopic microscope, with an ocular micrometer calibrated for 20x magnification. Three body size measurements were made to the nearest 0.01 mm on each weevil: left hind tibial length, pronotum width at its widest, and thorax plus abdomen length. It was not possible in most cases to measure the full body length of these biocontrol agents, as most weevils were preserved with the head and rostrum tucked into the pronotum. Additionally, total body mass was measured to the nearest 0.01 mg using a Mettler AT261 microbalance. The sex of each weevil was determined by observing external morphological characteristics of intact weevils under a stereoscopic microscope. Male weevils have a visible spur on the distal end of the hind tibia and a conspicuous denticle on the profemur (Appendix 1).

Data for each of the four body size measurements performed on adult *M. janthinus* (n=700 weevils) were normally distributed. Pearson product moment correlation analysis was performed to determine the association between the body length measurements and

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weevil body mass. The influences of weevil gender and year of release on each of the four body size measurements were explored using multiple linear regression. Although there may have been potential for movement of individual weevils between the various study sites (i.e. sites separated by at least one linear kilometre), grouping *M. janthinus* by time since weevil release made the assumption that populations at each of the release sites were independent of one another. Movement of weevils even within patches appeared to be minimal (V. A. Carney, personal observation). Sex ratios of weevils at non-outbreak and outbreak sites were compared using a chi square test for goodness of fit.

The influences of host availability and larval density on adult weevil size

Four sample sites were chosen for toadflax stalk collection, based on 2001 weevil counts at *M. janthinus* release sites (Table 1.1), to determine the levels of *M. janthinus* larval crowding within stalks. Low numbers of weevils were found at both of the Sand Creek Rd. sites: 6.1 weevils per minute at 0.5 Sand Creek Rd., and 13.6 weevils per minute at 2.0 Sand Creek Rd. By comparison, counts at ABH and Morrissey were 3-7 times higher, with 38.7 and 46.6 weevils per minute respectively. Stalks were subsequently pooled according to the size of the adult weevil counts. Two sample groups were compared: non-outbreak (Sand Creek Rd. 0.5/2.0) and outbreak (ABH/Morrissey).

In August 2001, an approximate total of 80 Dalmatian toadflax stalks containing fully developed pre-diapause adult *M. janthinus* were haphazardly collected from the two

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outbreak populations in Grand Forks, BC. The following May, an equivalent number of toadflax stalks containing post-diapause adults were similarly collected from the two non-outbreak populations. Weevils collected in stalks in August 2001 were artificially overwintered within the toadflax stalks in a 0°C chamber until dissection in February 2002. The post-diapause adults collected in May 2002 were held at 25°C until June 2002, when they were likewise removed from stalks. Both sets of Dalmatian toadflax samples contained weevils that would become the 2002 adult generation.

Sub-samples of the collected stalks were randomly chosen (30 from the outbreak sample, 72 from the non-outbreak sample) and the length of each measured. Stalks were split open to remove adult *M. janthinus*. A measurement of stem diameter, to the nearest 0.1 mm, was made at the base of each stalk using calipers. Since Dalmatian toadflax shoots senesce in late summer, stem diameter observations taken after this period provided a reasonable approximation of the host quality/size available to developing weevils at the final stages of larval and pupal development in 2001. The densities of weevils within toadflax stalks were calculated on a per cm stalk length basis.

Comparisons between sample groups of mean stalk length, diameter and amount of host resources available to individuals (measured as stalk length divided by the mean density of adult weevils per stalk) were made using Mann-Whitney U tests. Estimates of the linear length of toadflax stalk available per adult weevil are artificially inflated for both sample groups. This is because the shoot lengths available to all developing larvae are not reflected, rather to the percentage of a cohort that successfully competed to reach the

adult stage. It does, nonetheless, provide a relative estimate of the resource-limiting stress facing individual *M. janthinus*.

Linear measurements were performed on individual weevils extracted from toadflax stalks, to the nearest 0.01 mm, including: left hind tibial length, pronotum width, and thorax plus abdomen length. Additionally, body mass (mg) of fresh insects was measured. Multiple linear regressions were used to compare the effects of gender, sample group (outbreak vs. non-outbreak *M. janthinus* populations), stem diameter at the point of weevil removal, stem length and density of weevils within stems on individual weevil size. All body size measurement data had normal distributions and were, thus, left untransformed for analysis.

The relationship between female body size and reproductive fitness

A study was conducted between 12 February and 14 June 2002, using a sub-sample of the adult weevils contained in cold-stored Dalmatian toadflax stalks from outbreak-site stalk collections in August 2001. There was a wide range of adult body sizes observed at each of these field sites. Measured females from the weevil size/density study were paired with two conspecific males immediately upon removal from stalks and placed in individual mating chambers constructed from 1 L plastic tubs with mesh lids. Each mating group was given two robust stems of fresh toadflax in water-filled vials for food and oviposition. The cut end of each toadflax shoot was inserted through a tiny slit into a parafilm-covered water vial in order to prevent shoot wilting and weevil drowning.

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Mating containers were held in an environmentally-controlled growth cabinet at 22°C, 16L:8D photoperiod, and 50% humidity. Toadflax shoots were replaced every 3-4 days and inspected for oviposition scars. Shoots were dissected when scars were apparent to check for successful oviposition and to test whether the presence of oviposition scars could reliably approximate the level of oviposition in stems. Female weevils are synovigenic and were, consequently, allowed to lay eggs throughout their natural lifetime. Death of a female signified the end of the replicate. However, dead male weevils were replaced with conspecific males from the original population source throughout the study to ensure continuous mating. A total of 66 replicates were set up and 49 utilized in the analysis (17 replicates were discarded due to accidental female death).

Seventy randomly selected eggs from 15 replicate females were dissected from the fresh toadflax shoots over two sample dates, 19 and 25 March 2002. The length and width of each egg was measured and volume estimated using the formula for a prolate spheroid (Zwillinger, 1995):

$$Volume = \frac{4\Pi a^2 b}{3}$$

where a represents the equatorial radius of the spheroid, and b represents the polar radius. The association between egg volume and female parent body size was assessed using Spearman rank correlation analysis for each of the four adult weevil size measurements studied.

Associations between female parent body size and other reproductive fitness measurements also were evaluated using Spearman rank correlation analysis. Four

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reproductive parameters were tested against each body size measurement: potential

lifetime fecundity, length of the pre-oviposition period, oviposition duration and female

parent lifespan. Potential lifetime fecundity, as defined by Roitberg *et al.* (2001), was

measured by the total number of eggs laid by individual females during their lifespan

under laboratory conditions. Length of the pre-oviposition period and oviposition

duration were calculated from the setup date to the sample date of first oviposition, and

from the first oviposition to the date of the female's death, respectively. Female parent

lifespan was calculated by subtracting the date of setup from the date of female weevil

death.

All means presented for data in this paper are untransformed, accompanied by estimates

of sample standard error unless otherwise specified. Data were analyzed using Systat 10.2

and SPSS 10.0.

Results

Study populations

The abundance of adult weevils at release sites, measured as the mean number of adult

weevils counted per minute per site (Table 1.1), was predicted by the number of years

since the initial weevil release (Fig. 3.1). A total of 59% of the variation in weevil

abundance was explained by time since weevil release ($F_{8,91}=15.011$, $P<0.001$). There

was no difference in the pattern of adult weevil abundance between the sample years

($F_{1,91}=1.665$, $P=0.200$). In both years, peak weevil counts were observed at sites that had

been established for 4 years: 60.8 (±7.3 SE) weevils per minute in 2001, and 46.6 (±5.5

SE) weevils per minute in 2002. After 4 years post-establishment, a decline in adult

weevil abundance was observed during both sample years.

Size variation and sex ratios of field-collected *M. janthinus* adults

Correlation analysis showed that each of the chosen measurements was highly associated

with the others (Table 3.1), suggesting that each of the body size measurements should

display relatively consistent patterns of size variation. The mean mass of the field-

sampled weevil population, pooled across gender, was 4.66 ± 0.07 mg, ranging from 0.83-

11.20 mg (Fig. 3.2a). Thorax plus abdomen length ranged from 2.73-5.57 mm with a

mean of 4.18 ± 0.02 mm. Measurements for pronotum width ($\mu=1.25\pm0.005$ mm, range

0.75-1.64 mm) and left hind tibial length ($\mu=0.77\pm0.004$ mm, range 0.40-1.23 mm) had

relatively narrow sample variances, indicating that these measurements may be relatively

fixed across a range of adult weevil body sizes. Consequently, body mass and thorax plus

abdomen length are potentially more sensitive indicators of environmentally-induced

sources of variation.

Since the body size indicators are highly correlated with one another, and analyses

revealed similar patterns influencing and resulting from the various body size

measurements, details of the results for body mass alone are subsequently discussed. The

mass of adult weevils was significantly influenced by the year of weevil release and

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weevil gender (r^2=0.262, $F_{1,698}$=124.029, P<0.001) (Fig. 3.3). Separate, but similar

regression equations were produced by reduced linear models for male and female

weevils (Fig. 3.3). Patterns of mean body mass across release years show that there is a

reduction in body size as time post-release increases for both sexes (Fig. 3.3; Table 3.2).

There is also consistent sexual dimorphism across all body measurements, with female

weevils significantly larger than males regardless of time post-release (females: r^2=0.139,

$F_{1,452}$=73.162, P<0.001, males: r^2=0.218, $F_{1,244}$=67.880, P<0.001).

Sex ratios of collected weevils were determined and compared across weevil release

years (Fig. 3.4). The proportion of females in samples showed a greater departure from

the expected value of 0.5 as time since weevil release increased. Females at non-outbreak

sites (releases made in 1999-2001) comprised 59% of the pooled sample whereas the

pooled sample of weevils at outbreak sites (1994-1998 releases) was 68% female. A

comparison across sample groups showed that outbreak populations had a significantly

greater departure from the 1:1 sex ratio than the newly established, non-outbreak weevil

populations (X_1^2=4.716, P=0.030).

The influences of host availability and larval density on adult weevil size

Fully developed adult weevils were found within stems with diameters ranging from 2.3-

5.9 mm. Successful development has previously been reported in stems with diameters

greater than 0.9 mm (Jeanneret and Schroeder, 1992). Stems from the non-outbreak and

outbreak sample groups had similar shoot basal diameters (Mann-Whitney U=942.50,

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P=0.523), with a pooled mean diameter of 4.2±0.1 mm (Fig. 3.5). Mean stalk length from

the outbreak populations was 40% shorter than those collected from the non-outbreak

sites (Mann-Whitney U=1385.00, P<0.001) (Fig. 3.5). The mean density of adult weevils

in stalks from outbreak sites was 4.6 times greater than within non-outbreak stalks.

Successful weevils from the ABH/Morrissey sample group were required to develop

within one tenth of the shoot material available to individual weevils from the Sand Ck.

sites (Mann-Whitney U=1611.00, P<0.001) (Fig. 3.5).

Similar to the size variation patterns in adult *M. janthinus* reported from June field

samples, weevils removed from stalks within both sample groups in this study showed

sexual dimorphism (Table 3.3). Weevils from the crowded stalks belonging to the

outbreak sample group were smaller on average than those from the non-outbreak sites

(Table 3.3). The size of *M. janthinus* individuals from outbreak sites also was more

variable than that of weevils in the non-outbreak group, as measured by a one-tailed

variance ratio test ($F_{449,249}$=1.177, P<0.001). The sex of weevils, membership in the

respective sample groups, stem diameter and density of weevils within stems

significantly accounted for 35.5% of the overall variation in adult weevil body mass

(n=800, P<0.001). Since male and female weevil sizes were influenced by different

factors, the data were further sub-divided and re-analyzed to elucidate which factors were

the most important influences on male and female *M. janthinus* size.

From the multiple regression analyses, it is clear that membership within designated

sample groups was the most important determinant of female mass (r^2=0.163), with stem

diameter (r^2=0.033) having a small but positive significant effect (n=525, P<0.001)
(Table 3.4). Similarly in males, stem diameter significantly and positively influenced
(r^2=0.065) body mass. Males were found to be more sensitive than females to weevil
crowding in stems regardless of the source of the weevil population sampled. The density
of *M. janthinus* within stems accounted for the remaining (r^2=0.075) variation in male
mass (n=265, P<0.001) (Table 3.4).

The relationship between female body size and reproductive fitness

Oviposition scars were very conspicuous on the tender shoots used in this study.
Dissections confirmed that the presence of oviposition scars reliably estimated the level
of oviposition within stems. Of the 3041 oviposition scars counted during this study,
98.4% were associated with a deposited egg. Numerous scars that were created early in
the study, mostly within the first two weeks of set up, did not lead to oviposition, nor did
females hollow out the interior of the stem in preparation of egg deposition. These
'practice' oviposition attempts differed from scars that began to appear near the end of
the average oviposition period (60-90 days after setup). The latter scars included sections
within the interior of toadflax shoots that were hollowed out and consistent with the size
of a single *M. janthinus* egg.

Females began to lay eggs as early as 15 days after removal from toadflax stalks in which
they had overwintered. However, a pre-oviposition period of more than three weeks was
commonly observed (μ=24.9±1.6 days) under laboratory conditions. The duration of

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oviposition ranged from 0-87 days with a mean egg-laying period of 46.9±3.7 days.

There were only four females within this experiment that did not lay eggs during their

lifespan. These females were, however, still included in the analyses. The size range of

sampled females was 1.22-7.81 mg (μ=4.85±0.16 mg) (Fig. 3.2b) and lifespan of these

weevils ranged from 6-117 days with a mean of 68.7±1.6 days. During this period, an

average of 45.5±5.0 eggs were laid per female. One female achieved a maximum

potential fecundity of 118 eggs.

Potential fecundity was positively correlated with female lifespan (n=49, r_s=0.471,

P=0.001), indicating that longer-lived females achieved greater reproductive success.

Fecundity was significantly higher when the pre-oviposition period was shorter (n=49,

r_s=-0.556, P<0.001), but female weevil longevity was not affected by the duration of this

period (n=49, r_s=-0.234, P=0.142). The duration of oviposition is reduced as the length of

the pre-oviposition period is extended (n=49, r_s=-0.500, P=0.001). The cues that initiate

M. janthinus oviposition are unknown, however, mating was observed to begin almost

immediately after setup of the study and the first practice oviposition scar was apparent

within 2 days.

The volume of individual eggs (n=70) was constant across the range of female parent

sizes (Table 3.5), with a mean of 1.57±0.06 mm^3. This indicated that individual

reproductive effort of the sampled weevils is fixed despite the potentially variable vigour

of female parents. Furthermore, there was no relationship between the size of female

parents and any of the other reproductive parameters tested. Larger females did not live

longer, nor did they produce more eggs than smaller females within this study (Table 3.5). With the exception of a marginally significant relationship between thorax plus abdomen length and the length of the pre-oviposition period (r_s=298, P=0.049), neither the duration of the pre-oviposition period nor the actual oviposition period were influenced by female body size (Table 3.5).

Discussion

Field populations of *M. janthinus* in the Grand Forks, BC area displayed outbreak population dynamics (Fig. 3.1), a trait correlated to the success of an introduced weed biological control agent (De Clerck-Floate and Bourchier, 2000; Crawley, 1986). Studies of weevil abundance across the number of years since release show that *M. janthinus* population dynamics are characterized by increases in abundance towards peak or outbreak levels (20- to 80-fold increase in counts) within 4 years of release, followed by a decline in numbers at older release sites (Fig. 3.1). The outbreak population dynamics displayed by *M. janthinus* are shared by some well-known pest species, such as *Choristoneura fumiferana* (Clark *et al.*, 1979) and *Dendronoctus ponderosae* (Raffa and Berryman, 1983). These and other eruptive pest species, have several population patterns in common with *M. janthinus*, including the abilities to reach high population densities and cause high levels of host damage, along with fluctuating populations that may follow a cyclic or endemic-epidemic abundance pattern (Berryman, 1987).

Reduced host plant availability at peak *M. janthinus* densities may be a key regulatory factor in the weevils' population dynamics through negative feedback. Haukioja *et al.* (1983) hypothesize that the primary forces driving herbivore population cycles are extrinsic, relating to host plant availability and predation. Endophagous specialists, such as *M. janthinus*, are highly dependent on the quality and quantity of their host plants (Dodge and Price, 1991), as oviposition choices determine the resource levels ultimately available to their offspring. It has been shown that outbreak population levels of nursery-reared *M. janthinus* correspond with significant reductions in toadflax shoot biomass and reproductive output (Chapter 2). In this study, field observations (Fig. 3.5) indicate that sites with outbreak populations of *M. janthinus* show a 40% reduction in *L. dalmatica* stem length compared to stems at sites with low weevil populations. Similarly, Saner *et al.* (1994) found that a 30% reduction in above-ground Dalmatian toadflax biomass could be experimentally attributed to *M. janthinus* larval feeding within one growing season. Declining host quality under intense herbivory plays a larger role in reducing insect vigour, particularly in endophagous herbivores (Wheeler and Center, 1997), than do top-down effects of parasitism and predation in given systems (Hunter and Price, 1992). Neither of these effects was measured in the current study, but both warrant evaluation as contributors to the reduction of weevil population size over time. It is clear from this study, however, that *M. janthinus* can induce serious restrictions on the availability of its only host to successive weevil generations, and potentially initiate a delayed density-dependence within its population (Ylioja *et al.*, 1999).

Body size variation is widely believed to have a large non-genetic component (Alcock, 1984), primarily determined by the amount of food consumed during larval development (Juliano, 1985). There is a strong potential for *M. janthinus* to undergo some level of size-related selection under such intense levels of resource limitation. Weevil populations rapidly expand at release sites (up to 80-fold within 4 years of release), further contributing to the severe resource stress on weevil populations and increasing the potential for intense intraspecific competition amongst developing weevils. Clear patterns were observed of reduced weevil size as time passed since release, and correspondingly, as weevil abundance increased (Fig. 3.3). Not so clear, however, is whether this reduction in individual weevil size represents an adaptive response of *M. janthinus* to the decline in food and reproductive resources or that it is indicative of a potential reduction in weevil fitness. Sampled weevils show significantly greater size variability at outbreak sites, suggesting that there may be adaptation at the phenotypic level. Increased phenotypic plasticity as resources become increasingly limited benefits insects by allowing the population to adjust to the new environmental conditions (Myers, 1987).

Due to the protocol used in determining the relationship between host availability and insect size, it is possible that the differences in mean body mass between the two sample groups (outbreak and non-outbreak) could have been attributed to the length of time weevils from each group remained in stalks prior to stalk dissections. Weevils from the Sand Ck. (i.e. non-outbreak) group spent 3 months longer in diapause than those from the ABH/Morrissey (i.e. outbreak) group and would likely be required to use more fat reserves to sustain themselves as a consequence (Chapman, 1982). It would, therefore, be

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expected that the Sand Ck. weevils would have been smaller as a result of their prolonged diapause. Despite the disparity in overwintering duration between the sample groups, however, the pattern emerged of smaller weevils at the more densely populated yet earliest-dissected, outbreak sites. In the case of *M. janthinus*, mass is a sensitive enough size measurement to reflect the intensity of density-dependent stress on the weevil populations. There was little concern that the differences in sampling protocols affected the linear body size measurements of adult *M. janthinus*, as the weevils' cuticles becomes fixed in size during the final molt to adult stage (Wigglesworth, 1972). The sampling methodology did, however, prevent a comparison of sex ratios between the two sample groups, as it was not possible to differentiate the sex of most dead, overwintered adults. As a consequence, gender-related survival may have seriously influenced the proportion of females found in spring stalk samples. Another stalk collection from both site types in spring would be required to test whether the secondary sex ratio of *M. janthinus* is skewed by reduced overwintering survival of males at outbreak sites.

Results from the fecundity study showed no correspondence between female insect size and the reproduction parameters tested (i.e. lifetime egg production, adult lifespan, duration of the pre-oviposition and oviposition periods, and size of individual eggs produced). This suggests that *M. janthinus* does not appear to be self-regulating or compensating for high population densities (Haukioja *et al.*, 1983). This is contrary to the bulk of studies published on the size-reproductive fitness relationship (Bellinger *et al.*, 1990; Credland *et al.*, 1986; Palmer, 1985). Most of the literature attributes reduced fecundity to the decrease in insect size as a result of negative environmental change,

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frequently referred to as the compensation hypothesis (Haukioja *et al.* 1983). However, in a review of the subject, Leather (1988) states that "...acceptance of the conventional tenet that big insects are more fecund than small ones is not a viable proposition...", suggesting that detailed knowledge of all factors affecting fecundity are required to accurately predict how reproductive fitness is affected by variation in insect size. It is possible that any underlying relationship between the size and fecundity of this biocontrol agent may have been masked under an ideal feeding regime, as female *M. janthinus* develop and produce eggs continuous through their lifetime. Klingenberg and Spence (1997) hypothesized that the key advantage of greater size (i.e. greater body reserves) one might expect to see in food-limited situations may not be revealed under ad libitum feeding conditions. Their results of fecundity testing under two different food levels, however, refuted the prediction and showed that size did not provide any reproductive benefit to female *Gerris buenoi*. Results from this study, and those of Klingenberg and Spence (1997), agree with Leather (1988) that there is not necessarily a direct cause-and-effect relationship in insects between female body size and reproductive success.

The impact of size reduction on the fitness of *M. janthinus* is more subtle than can be discerned using the parameters tested in this study. Potentially negative consequences of reduced size in a resource-stressed population include decreases in overwintering survival (Palmer, 1985), fecundity (Credland *et al.*, 1986), mobility and dispersal (Nylin and Gotthard, 1998) and adult longevity (Quiring and McNeil, 1984), particularly if body size determines an insect's nutrient reserves (Ohgushi, 1996). Conversely, smaller insects may be displaying successful adaptation to limited resources (Nylin and Gotthard, 1998)

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and achieving improved male mating success as a result of shortened development time (Price, 1997). More difficult to measure are the indirect size influences on factors such as female mating fitness (Scheiring, 1977) and oviposition success. The ability of endophagous insects to oviposit on choice stem parts, for example, may be compromised if females are too small to hold and chew holes into the tough epidermis of host plant stems (Eber *et al.*, 1999). Relationships between size and the fitness indicators mentioned above, with the exception of fecundity, have yet to be determined within the *M. janthinus* – *L. dalmatica* system. Measurement of the association between size and these additional factors would provide a more comprehensive explanation of how populations of *M. janthinus* are influenced by the increase of density-related resource stress.

It is possible that the lack of association between size and reproduction may be an artifact of the selection of females available for use within this experiment. The sampling distribution of mass of females used for this experiment had a very narrow interquartile range compared to females sampled across 14 study sites (Fig. 3.2). Replication of this experiment using a range of female sizes from both outbreak and non-outbreak sites is needed to elucidate the results of this study.

The implications of a shift in *M. janthinus* sex ratio toward females at higher levels of resource stress are unclear at present. Fisher's Principle (Hamilton, 1967) predicts that large, randomly mating populations should have a sex ratio that approximates 1:1. During high levels of reproduction, however, some insect populations show a highly female-skewed sex ratio (Dix and All, 1985). This can be achieved during periods of intense

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local resource competition by either facultative manipulation of offspring sex ratios by female parents (Varndell and Godfray, 1996), or by differential survival of offspring contributing to a changed secondary sex ratio (Cole, 1973; Ishihara and Shimada, 1993). The latter explanation may be more applicable to *M. janthinus* sex ratio dynamics, as male weevil size was shown to be more sensitive to crowding than that of females, and males were found to be significantly smaller than conspecific females. Comparisons of sex ratios showed fewer male weevils under crowded conditions (Fig. 3.4). Differential survival by gender, particularly as overwintering success, may explain the skewed sex ratio under variable levels of resource limitation. Alternatively, differences in *M. janthinus* dispersal behaviour may be responsible for altering the secondary sex ratio in crowded toadflax patches. It remains unknown whether *M. janthinus* females can facultatively alter primary offspring sex ratios under different levels of resource stress, as is frequently seen in galling Hymenoptera (Craig *et al.*, 1992).

It is clear that *M. janthinus* shows a strong density-dependent response to environmental changes, particularly those relating to reductions in food and habitat availability. It has not yet been shown whether decreased size of *M. janthinus* adults represents an adaptive trend or signals a declining fitness. Sex ratios show a significant response to environmental variation, but interpretation of this trend also remains unclear until further study is done. This study has clearly shown, however, that there is no obvious or direct relationship between environmentally-induced size variation and fecundity of female weevils. If fecundity were found to be contingent upon female size, recommendations for biological control practitioners would have included harvesting weevils from adequately

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populated, pre-outbreak sites rather than collecting weevils of low vigour from high-density populations. The number of *M. janthinus* released at individual sites in BC (200-600) has, so far, been adequate to achieve successful establishment, despite the evidence from this study that a random collection of insects from different nursery sites would not yield identical insect populations, nor would it provide consistent sex ratios. Studying the effects of weevil density on other key measurements of insect fitness, i.e. survival to adulthood and rate of development within stems, in addition to the size-related parameters tested in this study, would answer many of the remaining questions about the long term sustainability of outbreaking populations of this biological control agent.

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TABLE 3.1. Summary of associations among adult *M. janthinus* body size measurements; adults taken from 14 study sites sampled in June 2002. Pearson product moment correlations are reported.

	Thorax+Abdomen Length	Hind Tibial Length	Pronotum Width
Body Mass	0.790	0.577	0.705
Pronotum Width	0.817	0.702	
Hind Tibial Length	0.692		

TABLE 3.2. Effects of gender and the number of years since weevil release on the size of adult *M. janthinus*. Data for the full (df=1,698) and reduced (males: df=1,244, females: df=1,452) regression models are presented below for each linear body size measurement. Slopes and intercepts are given for the reduced models only.

Size Measurement	r^2	F	P	slope	y-intercept
thorax plus abdomen length (mm)	0.207	90.722	<0.001		
male	0.041	19.139	<0.001	-0.0429	4.126
female	0.066	17.364	<0.001	-0.0371	4.484
left hind tibial length (mm)	0.111	43.595	<0.001		
male	0.048	12.386	0.001	-0.0101	0.774
female	0.025	11.791	0.001	-0.0079	0.834
pronotum width (mm)	0.157	65.105	<0.001		
male	0.049	12.669	0.001	-0.0013	1.235
female	0.022	10.348	0.001	-0.0089	1.328

TABLE 3.3. Comparison of adult weevil sizes between the non-outbreak and outbreak sample groups, reduced by sex. Mean size measurements are presented per stalk, accompanied by standard errors and ranges. The sample size reported represents the total number of weevils dissected from stems within each sample group.

	n	Mass (mg)	Thorax + Abdomen Length (mm)	Pronotum Width (mm)	Tibial Length (mm)
Non-outbreak:					
male	58	4.44±0.18 (1.32-7.82)	3.2±0.30[a] (0.4-8.6)	1.3±0.02 (0.9-1.5)	0.8±0.01 (0.5-1.0)
female	293	5.78±0.07 (1.53-8.99)	2.6±0.11 (0.4-8.2)	1.4±0.01 (0.9-1.5)	0.9±0.01 (0.5-1.0)
Outbreak:					
male	207	3.89±0.06 (1.08-7.21)	2.9±0.02[a] (1.08-7.21)	1.2±0.01 (1.3-4.4)	0.7±0.01 (0.6-1.4)
female	242	4.79±0.07 (1.22-8.83)	3.1±0.01 (1.8-3.5)	1.3±0.01 (0.9-1.5)	0.7±0.01 (0.5-0.9)

[a] not significantly different across the selected body size measurement at $P=0.05$

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TABLE 3.4. Host availability factors influencing *M. janthinus* mass, as determined by multiple linear regression analysis. Values displayed were generated from separate analyses for male (n=265) and female (n=535) weevils. ß represents an un-standardized slope.

	r^2	ß	*P*
Females:			
stalk diameter	0.033	0.177	0.000
sample group	0.163	-0.773	0.000
stalk length		0.001	0.170
weevil density		-0.394	0.272
Males:			
stalk diameter	0.065	0.257	0.000
sample group		0.027	0.898
stalk length		0.002	0.313
weevil density	0.075	-1.327	0.001

TABLE 3.5. Spearman rank correlations between female *M. janthinus* body size measurements and experimentally-determined fecundity parameters from 49 replicate females.

	Mass (mg)		Thorax+Abdomen Length (mm)		Hind Tibial Length (mm)		Pronotum Width (mm)	
	r_s	P	r_s	P	r_s	P	r_s	P
Potential Fecundity	-0.047	0.750	0.075	0.615	-0.007	0.963	-0.030	0.838
Adult Lifespan	-0.080	0.603	-0.148	0.333	0.014	0.926	-0.104	0.496
Pre-oviposition Period	0.040	0.797	0.298	0.049[a]	0.135	0.382	0.247	0.106
Oviposition Duration	-0.157	0.297	-0.201	0.181	-0.182	0.227	-0.229	0.126
Egg Volume	0.035	0.774	0.047	0.709	0.014	0.905	0.067	0.583

[a] correlation is significant at the 0.05 level

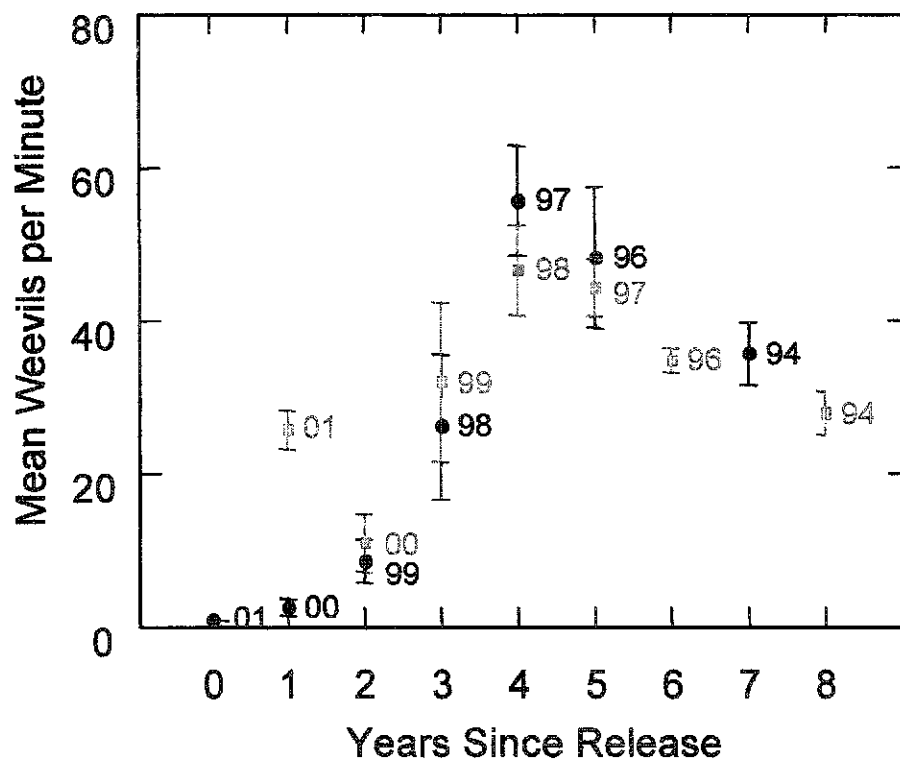FIGURE 3.1. Comparison of adult weevil counts in 2001 (●) and 2002 (▦) across 17 study sites (Table 1.1), grouped by the number of years since initial weevil release. Mean counts per minute ± 1 SE are presented, with the year of release recorded beside each mean. Sample sizes for release years (n= number of sites) are: 1994 (3), 1996 (1), 1997 (3), 1998 (2), 1999 (3), 2000 (4), 2001 (1).
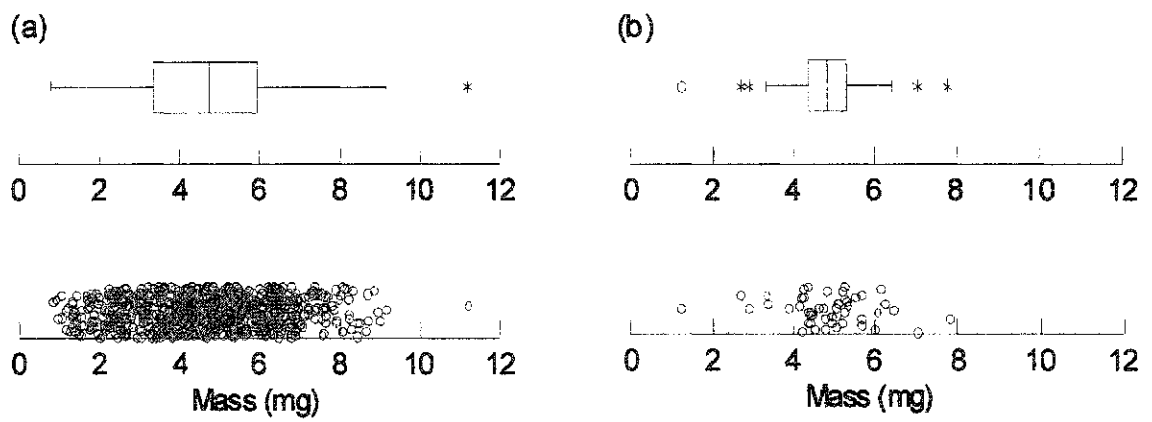
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FIGURE 3.2. Comparison of the size distributions of adult *M. janthinus* (a) sampled from 14 study sites (n=50 per site), and (b) used in the size vs. reproductive fitness evaluation (n=49 total). The central tendencies of data are presented in box plots (above) and the spread of data in jittered dit plots (below).
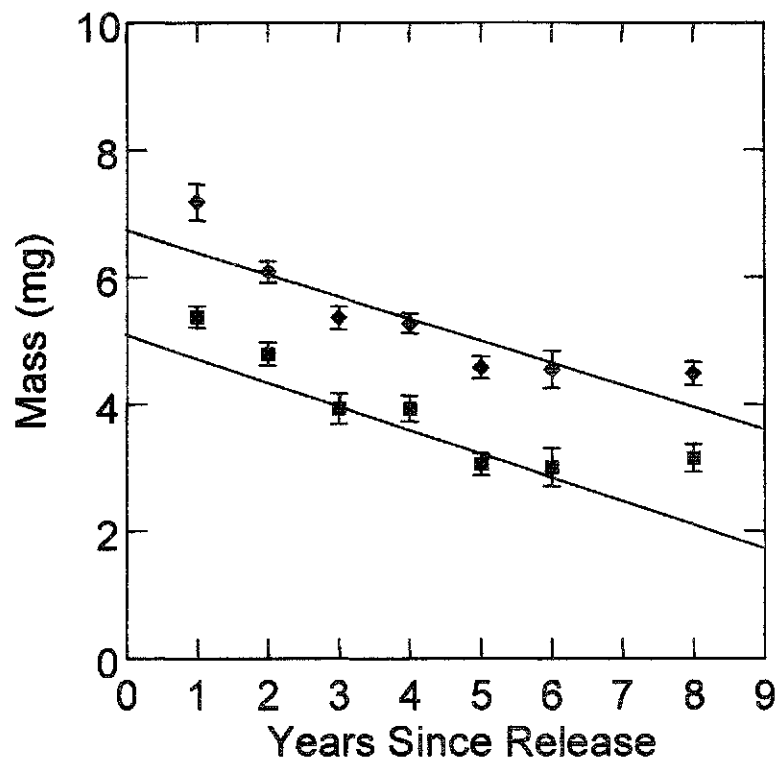
FIGURE 3.3. Reduction of adult *M. janthinus* body mass by time since initial release. Means ± SE are shown for each of the years since weevil release along with lines of best fit for the linear regressions, n=454 females (◆) and n=246 males (■). Regression equations are: y=6.511-0.297x (female) and y=5.145-0.303x (male), where y and x represent body mass (mg) and years since release, respectively.

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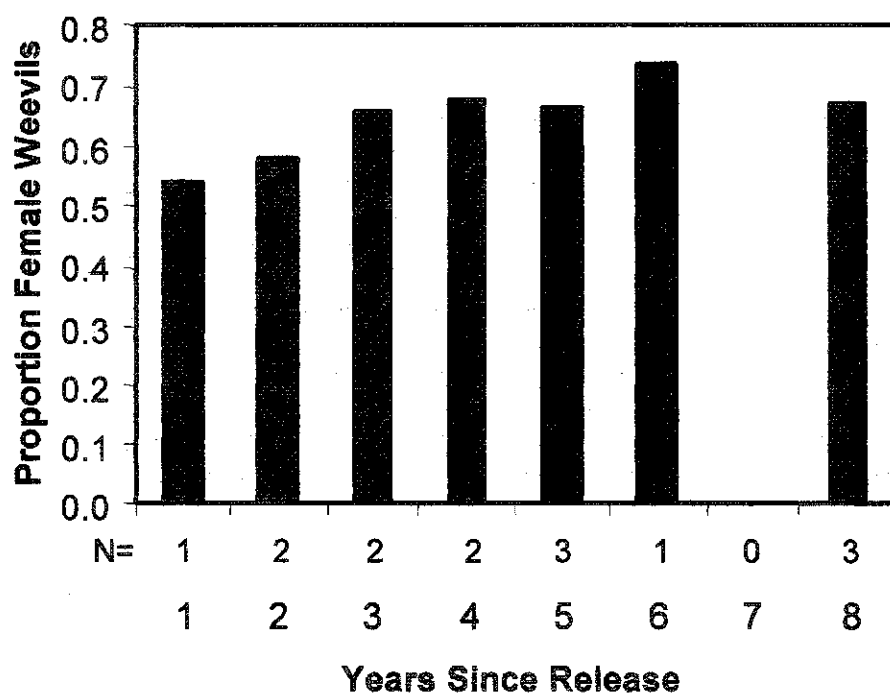
FIGURE 3.4. Comparison of adult *M. janthinus* sex ratios across time since initial release. The number of sites studied from each release year (n) is listed below the x-axis. Sex ratios were determined from a sample of 50 weevils collected per site.
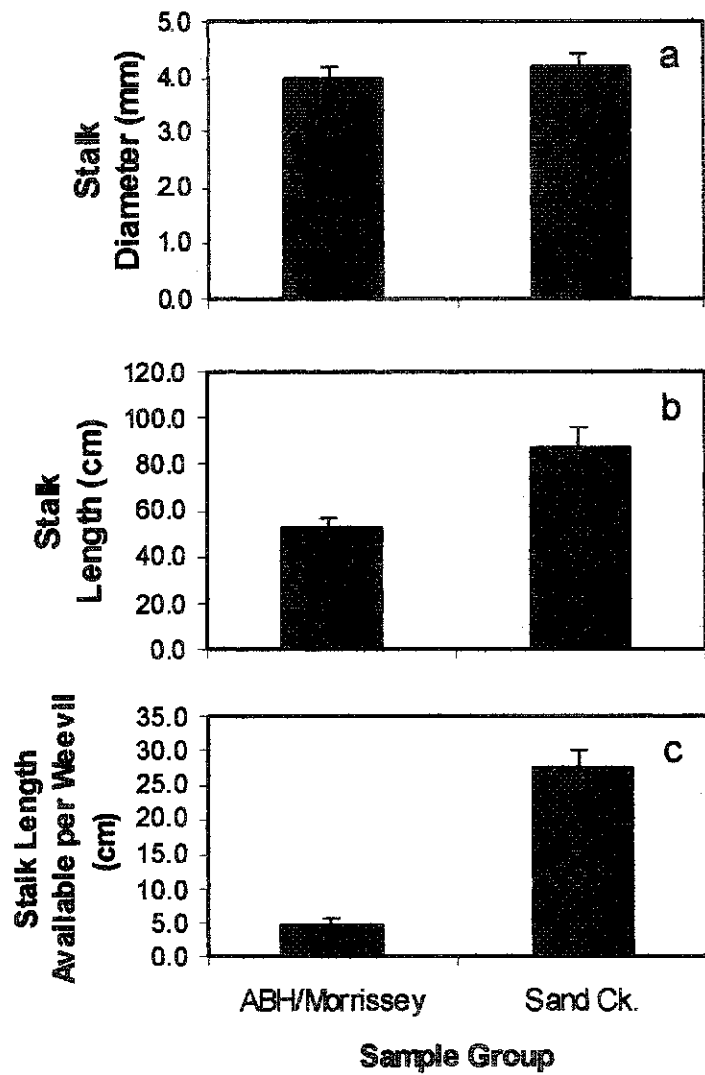
FIGURE 3.5. Patterns of (a) stalk diameter, (b) stalk length, and (c) the amount of linear toadflax shoot length available to developing *M. janthinus*. Bars represent means +SE. The number of stems sampled from the ABH/Morrissey sites (i.e. outbreak) and the two Sand Ck. sites (i.e. non-outbreak) were 30 and 72, respectively.

CHAPTER 4

Assessing the strength of association between oviposition preference and offspring performance

Introduction

Examining the relationship between host selection and offspring performance is key to developing theory on herbivore-plant interactions (Mayhew, 1997, 1998; Price, 1991, Thompson, 1988). The resource choices made by ovipositing female insects have direct influence on the success of future generations and, in turn, define how populations respond to both spatial and temporal resource heterogeneity (Ohgushi, 1995). One hypothesis states that phylogenetic constraints placed on individual insect species cause an adaptive syndrome of characters to evolve that minimize the effects of such constraints and maximize the effective use of hosts (Price et al., 1995). For example, shoot boring, galling and mining insects are constrained to oviposit into growing plant tissues (Price et al., 1990) where larvae have limited mobility and little opportunity to choose optimum resources for development (Valladares and Lawton, 1991). One adaptive response to this constraint involves the evolution of the ability of females to differentiate and seek high quality resources for oviposition (Koricheva and Haukioja, 1994), thus improving the success of offspring.

There has been a wide variation in the level of correspondence between host selection and offspring performance of insects in the literature (Valladares and Lawton, 1991).

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Thompson (1988) reviews many of the early papers and hypothesizes on reasons for a

lack of correspondence between preference and performance. Many studies involving

mobile defoliating insects reported no correspondence between host choice and offspring

success (Kimberling and Price, 1996; MacKay, 1995; Rausher, 1979; Yee and Toscano,

1996), and some found that females of these insects were not selective in choosing

oviposition sites (Gannon and Bach, 1996; Mappes and Kaitala, 1995). Recent studies

have examined the preference-performance relationship in endophagous herbivore

populations, such as leafminers (Eber *et al.*, 2001; Koricheva and Haukioja, 1994), stem

and leaf gallers (Craig *et al.*, 1989; Dodge and Price, 1991; Eliason and Potter, 2000;

Horner and Abrahamson, 1992; Pires *et al.*, 2000; Stein and Price, 1995), stem borers

(Shibata *et al.*, 1994), and seed feeders (Briese, 1996; Fox, 1993). The majority of these

studies reported a tight association between host selection by ovipositing females and

larval performance. In environments with heterogeneous host resource quality,

endophagous insects undergo intense selection to optimize oviposition site selection

(Stein and Price, 1995). In species with reduced larval mobility and searching ability,

oviposition behaviour drives the population dynamics of those insects (Ohgushi, 1995). A

female must choose high quality resources or her offspring will not survive.

A strong correlation between oviposition site selection and offspring performance is

expected for insects with latent (or non-eruptive) population dynamics. Because females

of latent species have strong preferences for high quality resources (Price *et al.*, 1990),

which are often rare in nature, herbivore populations are kept at low, stable levels (Levya

et al., 2000). Eruptive species are those with both relatively stable dynamics under low

densities and epidemic phases, in which population densities are high and insects are particularly destructive to their hosts (Dodge and Price, 1991). Available studies support the view that there is little correlation between preference and performance in eruptive species (Dodge and Price, 1991; Legg *et al.*, 1986; and Levya *et al.*, 2000). It is hypothesized that females of eruptive species cannot discern the quality of resources (Price *et al.*, 1990), thereby not developing distinct host preferences. However, few studies have evaluated the preference-performance relationship in eruptive species in detail and even fewer have looked at insects that are endophagous with eruptive tendencies.

Mecinus janthinus is a stem-boring weevil that achieves outbreak densities, during which weevil populations severely affect their host plant (Chapter 2). At sites in Southern British Columbia (BC), weevils are confronted with choices between different shoot morphologies on which to lay eggs. The main goal of this chapter was to test the strength of the oviposition preference-offspring performance relationship under different population densities in both laboratory and field settings, along with determining whether parent larval experience influences host selection. This system provides an unique opportunity to evaluate the host choices made by an endophagous insect and their consequences on offspring success during two different phases of this species' eruptive dynamics.

Methods

Field survey of *M. janthinus* shoot choice and larval survival

Dalmatian toadflax grows from seeds, taproots and vegetative spread of adventitious root tissues (Alex, 1962). A single plant produces between 1-25 floral shoots (Robocker, 1974) and 3-40 short, sterile offshoots annually (Vujnovic and Wein, 1997). Hence, within a patch of toadflax, adult *M. janthinus* are presented with different shoot morphologies for feeding and oviposition. To determine if ovipositing *M. janthinus* are selective across the various available toadflax shoot types, shoots were arbitrarily classified into 3 categories: short vegetative (single, non-flowering shoots shorter than 25 cm), tall vegetative (individual, non-flowering shoots taller than 25 cm) and reproductive (flowers or flower buds apparent, tall and generally with lateral branches along the primary raceme). Inclusive in the short vegetative shoot category are both sterile offshoots of a mature toadflax plant and seedlings. Between 18-22 June 2001, ten shoots of each type were haphazardly collected from within a 20 m radius of initial *M. janthinus* release points at 12 study sites near Grand Forks, BC (see Table 1.1). Six of these study sites contained weevil populations categorized as outbreaking, with a median adult weevil count of 40.1 per minute and range of 27.4-48.2 weevils per minute (n=2 counts per site). The other six study sites contained non-outbreak *M. janthinus* populations, with a median adult count of 6.5 per minute, range of 0-15.6 weevils per minute (n=2 counts per site). Collected shoot material was held at 5°C and individual shoots were dissected through the end of July 2001. The total linear length per shoot (i.e. including the primary

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stem plus lateral branches) and length of each primary stem were measured to the nearest 0.5 cm, the basal shoot diameter measured to the nearest 0.1 mm and the number of lateral branches on each shoot was recorded. Estimates of the frequencies of reproductive versus vegetative shoots at both non-outbreak and outbreak study sites were made from quadrat surveys at the 12 study sites, using mean proportions of shoots of each type present within 30 quadrats, as described in Chapter 2. Examination of toadflax shoots for *M. janthinus* oviposition was made by counting external oviposition scars. An advantage to studying endophagous borers, such as *M. janthinus*, is that each oviposition event can be followed to its individual end by dissecting toadflax shoots during the growing season and identifying individual larval tunnels within the plant tissues. To standardize the host choice data, only the primary stems were examined for oviposition. Lateral branches are rarely attacked by *M. janthinus* (De Clerck-Floate and Miller, 2001).

As shoots were dissected, the presence of each external oviposition scar was compared to the weevil stage found within shoots in order to gauge both oviposition preference and performance of developing offspring. The term "oviposition preference" used in this paper follows Singer's (2000) hypothesis that there is a non-random, hierarchical order by which females utilize resources offered simultaneously for oviposition. Electivity describes the proportion of different shoot types used for oviposition relative to what is actually found in toadflax patches (Singer, 2000). The electivities of female *M. janthinus* among potential oviposition sites were measured in two ways: by assessing the relative frequencies of each shoot type attacked (i.e. incidence of attack), and comparing the relative densities of eggs laid into the three shoot types (i.e. intensity of attack). Shoot

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attack frequencies were evaluated between vegetative (short and tall combined) and reproductive shoot types individually by site category (outbreak and non-outbreak) using chi square tests for goodness of fit. The expected proportions of vegetative and reproductive shoots within sample populations were taken from shoot count data (Chapter 2), combined for site type, as presented in Table 4.2. Weevil densities were calculated for each of the three shoot types using the number of ovipositions per cm shoot, then square root transformed to meet normality assumptions (Zar, 1999). A GLM ANOVA was used to determine whether the intensity of weevil attack varied across site and shoot types. The full ANOVA model was subsequently reduced by outbreak category and oviposition differences between shoot types were determined using Tukey HSD adjusted-pairwise comparisons.

A gross measurement of offspring performance within the three shoot types was made using larvae developing within the collected toadflax shoots. For this, the proportions of larvae of all stages surviving to 26 July 2001 were calculated. Survival data were rank transformed due to lack of normality (Conover and Iman, 1981). Relative estimates of offspring survival were contrasted across site outbreak categories and shoot types using a GLM ANOVA on the rank transformed data, followed by Tukey HSD-adjusted pairwise comparisons by site category. A Spearman rank correlation was performed using all attacked shoots to assess the strength of association between oviposition electivity and larval performance.

Testing the oviposition preference – offspring performance relationship

A greenhouse experiment was set up to test if oviposition preferences by shoot type are exhibited regardless of shoot availability, and whether these choices are associated with offspring performance. The experiment also was set up to determine if the source of the adults (i.e. from crowded or uncrowded larval conditions within stems) or their density at the time of oviposition influenced shoot type choices. On 17 June 2002, fourteen replications of four treatments were set up. The experimental design was a 2x2 factorial with weevil source population (outbreak versus non-outbreak) and level of adult crowding (three versus 15 male-female pairs) as factors.

Dalmatian toadflax plants were started from seed in June 2001, transferred to 10 cm-diameter pots, and maintained under greenhouse conditions (daily watering, temperature of 22 ± 5 °C, 16L:8D photoperiod) until the beginning of the experiment. Individually potted toadflax plants were trimmed to within 3-5 cm of soil level on 27 May 2002 to standardize the age of shoot tissues available to ovipositing *M. janthinus*. One week prior to experimental set-up, 56 plants were chosen out of an available 82 and shoot regrowth was selectively trimmed so that weevils were presented with equivalent total lengths of vegetative and reproductive shoots. The experiment was set up in the same greenhouse under the previously described conditions. Toadflax pots were regularly spaced along the greenhouse benches and weevil treatments were randomly assigned to each pot. Twenty toadflax plants were randomly checked to ensure that the total shoot length per shoot type was similar across the experimental groups. Basal shoot diameters also were taken from

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just above soil level on a randomly selected shoot of each type within each replicate plant. Each pot was caged with a mesh sleeve on 17 June 2002 and weevils added according to assigned treatments. Sleeves were approximately 40 cm in diameter and 45 cm high, held upright with garden stakes.

The adult weevils used in this experiment were removed from toadflax stalks collected from field sites (2 outbreak sites, 2 non-outbreak sites) near Grand Forks, BC in early May 2002. The sex of each weevil was determined (see Appendix 1) and sexes were held separately until enough weevils became available for all treatment replicates. Individual weevil treatment replicates were held in small, plastic mating containers with a continuous supply of fresh toadflax as food from 24 May to 17 June 2002. This was done to prevent excessive feeding damage to experimental plants during the pre-oviposition period, which has been observed to be approximately 25 days at 22°C (Chapter 3). Upon experimental set-up, weevils were allowed to feed and oviposit for 15 days. On 2 July 2002, all weevils were removed from the experimental pots and oviposition scars were counted and marked with permanent ink. Plants were returned to the greenhouse bench without sleeves to resume *M. janthinus* offspring development. On 18 September 2002, all toadflax shoots were clipped at soil level and labeled by shoot type and experimental replicate. Shoots were dissected throughout September and October. All weevil stages, alive and dead, within shoots were counted. The sex of each adult *M. janthinus* was determined under a Leica MZ9.5 dissecting microscope and individuals were weighed to the nearest 0.01 mg using a Mettler AT261 microbalance.

Shoot characteristics (total shoot length available to the nearest 0.1 cm, basal shoot diameter to the nearest 0.1 mm, number of shoots per experimental type) were compared across the experimental replicates prior to set-up using a t-test or Mann-Whitney U test, depending on which characteristics met normality assumptions.

Shoot preferences of ovipositing females were measured as the densities of oviposition scars on vegetative versus reproductive shoots. Each oviposition scar present was considered a successful oviposition event (see Chapter 3). Egg densities were compared across shoot types to determine if preferences varied according to the source of parent weevils (outbreak vs. non-outbreak) and adult crowding levels (3 mating pairs vs. 15 mating pairs). Egg densities were calculated per cm shoot length and log_{10} transformed to meet the normality assumptions of analysis using a GLM ANOVA, followed by Tukey HSD adjusted-pairwise comparisons.

Offspring performance was assessed across experimental treatments using three fitness-related measurements (Roitberg et $al.$, 2001): proportion of offspring surviving to adulthood, an indirect measure of development rate using the proportion of total offspring that survived to each life stage, and the size of individual adult offspring. All performance measurements were compared across shoot types, source populations and crowding levels using GLM ANOVAs, with Tukey HSD adjusted-pairwise comparisons where necessary. Data were transformed in some cases to meet the normality requirements of this statistical test (Zar, 1999). Weevil gender was included in the analysis on weevil size, as it had been previously determined that there is significant

sexual dimorphism within *M. janthinus* populations (Chapter 3). Spearman correlation

analyses were performed to assess the strength of association between weevil densities

within shoots and offspring performance measurements.

Data analyses were performed using Systat 10.2 and SPSS 10.0. Untransformed means

are reported with standard error calculations unless otherwise specified.

Results

Field survey of *M. janthinus* shoot choice and larval survival

Reproductive shoots at field sites were larger than vegetative shoots, with taller primary

stems and thicker basal shoot diameters (Table 4.1). Short vegetative shoots, many of

which are young, delicate seedlings or weak offshoots (Vujnovic and Wein, 1997), had

few, if any, lateral branches. Nor do tall vegetative shoots, but reproductive shoots had up

28 branches, increasing their total linear shoot length substantially. This would

potentially improve the apparency of these shoots within a toadflax patch, even if lateral

branches were rarely attacked (Table 4.1). Typically, vegetative shoots, short or tall, were

distinct in patches of toadflax as upright, singular stems representing over 85% of the

total number of shoots within Dalmatian toadflax stands (Table 4.2). In contrast,

reproductive shoots, despite their bushy appearance and thick stems, comprised less than

15% of the shoots within a patch of toadflax (Table 4.2). The availability of each shoot

type for *M. janthinus* oviposition and feeding was determined by multiplying the mean

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total primary shoot length of the each of the two types by their respective representations within toadflax stands (Table 4.2). According to the indices of availability, vegetative shoot material would generally be encountered more frequently by ovipositing weevils at both non-outbreak and outbreak sites.

The incidence of weevil attack on toadflax shoots was much lower at sites where *M. janthinus* populations had not yet reached outbreak population levels (X_1^2=111.237, *P*<0.001). Almost every shoot collected from outbreak sites had some level of attack (99.4%), whereas only 51.7% of the shoots collected at non-outbreak sites had oviposition scars. The goodness of fit test from outbreak sites showed that the sole deviation from expected attack was due to the increased incidence of oviposition into reproductive shoots, which were 31 times more frequently used by *M. janthinus* females than expected under random oviposition (X_1^2=1756.93, *P*<0.001). This pattern of oviposition was consistent with observations made at sites with low insect densities (i.e. non-outbreak sites). Vegetative shoot attack was only 49% of what would be expected if weevils were laying eggs into randomly encountered shoots, whereas weevils were 4.7 times more likely to lay eggs into reproductive shoots than if they selected potential host shoots based on random encounter (X_1^2=143.55, *P*<0.001).

The densities of eggs laid into shoots were significantly higher at outbreak sites than non-outbreak ($F_{1,354}$=654.435, *P*<0.001). Oviposition densities also varied across shoot types ($F_{2,354}$=14.844, *P*<0.001) in the full GLM ANOVA model (r^2=0.661, n=360 shoots sampled). The interaction effect between site category and shoot type was not significant

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($F_{2,354}=2.416$, $P=0.091$). Reduced models by site category showed similar *M. janthinus* attack patterns across shoot types (Fig. 4.1). Tall vegetative shoots had significantly higher oviposition densities than reproductive shoots at non-outbreak sites ($F_{2,177}=7.433$, $P<0.001$) and more densely packed with eggs than either short vegetative shoots or reproductive shoots at outbreak sites ($F_{2,177}=11.379$, $P<0.001$). The outbreak status of the sample sites explained 44.8% of the variation in oviposition density within shoots, whereas shoot type was responsible for the remaining 7.4%.

The majority of *M. janthinus* found within toadflax shoots from all sites were early (i.e. first and second) instars. Larval survival was observed to be significantly higher in shoots from non-outbreak sites than within crowded shoots at outbreak sites ($F_{1,231}=4.199$, $P=0.042$). The ANOVA model ($r^2=0.112$, n=237 larvae) also detected a pattern of significantly reduced larval survival (~20% fewer offspring surviving) in short vegetative shoots ($F_{2,231}=8.520$, $P<0.001$) that was consistent across site categories (Fig. 4.2). Hence, the interaction between site category and shoot type was not significant ($F_{2,231}=0.120$, $P=0.887$). No significant correlation between oviposition density within toadflax shoots and subsequent larval survival was detected (Fig. 4.3).

Testing the oviposition preference – offspring performance relationship

The experiment was designed so that equal total lengths of reproductive and vegetative shoots were offered to weevils in each replicate to determine whether there was a distinct pattern of preference for one shoot type over the other. Adult *M. janthinus* were presented

with 77.9±12.3 cm of reproductive shoot length and 77.0±14.0 cm of vegetative tissues (mean±SD, n=20, Mann-Whitney U=204.5, df=1, P=0.903). Reproductive shoots were, on average, larger and more robust than vegetative. In the same random sample, it was observed that 5.3±1.1 reproductive shoots equaled 10.9±2.0 vegetative shoots to standardize for equal total shoot length. Within the total 56 experimental replicates, the basal diameter of reproductive shoots was 2.5±0.5 mm compared to that of vegetative, 2.1±0.6 mm (t_{pooled}=3.902, df=110, P<0.001).

The origin of adult weevils did not significantly influence the densities of eggs laid into shoots ($F_{1,104}$=1.114, P=0.294), indicating that the severity of larval crowding and intraspecific competition experienced by parent $M.$ $janthinus$ does not alter subsequent female preferences of oviposition sites. The other two factors tested in the model (n=112 plants, r^2=0.767), adult weevil density and shoot type, significantly influenced the density of eggs laid into shoots (Fig. 4.4). Reproductive shoots were preferred by females in each treatment, with approximately twice as many eggs being laid into reproductive versus vegetative shoots across all treatment combinations ($F_{1,104}$=51.336, P<0.001). Shoot type preference accounted for 32% of the overall variation in egg density within shoots. As would be expected, the treatments with 15 parent weevil pairs laid significantly more eggs than those with three adult pairs (Fig. 4.4) ($F_{1,104}$=289.463, P<0.001). There were no significant interactions between the factors.

The primary measurement of interest of offspring performance in this study was survival to the adult stage. Data for the proportion survival to adulthood were normally distributed

and, thus, analyzed untransformed within the ANOVA model (r^2=0.195, n=112

treatment/shoot type combinations). As with the preferences exhibited in this experiment,

the origin of adult mating populations did not have a significant influence on offspring

survival ($F_{1,104}$=0.640, P=0.425). There was differential survival across shoot types

($F_{1,104}$=7.906, P=0.006), corresponding to female shoot preferences. Over 10% more

developing weevils survived to adulthood in the reproductive shoots, despite the

increased levels of offspring crowding in these preferred shoots. There was reduced

offspring survival as a result of the higher adult density treatments ($F_{1,104}$=15.017,

P<0.001). None of the interactions between factors were significant. Figure 4.5 illustrates

the levels of weevil survival to the adult stage across shoot type and adult density

treatment. Adult weevil origin was omitted from this graphical representation as the mean

proportions of offspring surviving to adulthood for non-outbreak and outbreak weevils

were statistically identical (μ=0.375±0.028 and μ=0.347±0.026, respectively). Patterns of

offspring survival are consistent with female preferences (i.e. greater survival within

reproductive shoots). The model only accounted for 19.5% of the overall variation in the

proportion of offspring surviving to the adult stage, with shoot type as the major

contributor to the model (12.6%). There was a near-significant, negative correlation

between oviposition preference, as measured by egg density, and offspring survival when

all treatments were combined (Fig. 4.6a).

The mean proportions of total offspring within toadflax shoots represented by different

life stages (larvae, pupae, adults) were arcsine square root transformed prior to analysis,

1999). The full ANOVA model (r^2=0.313, n=336 treatment/life stage combinations)

tested for differences in mean proportions of representative offspring stages across parent weevil crowding history (non-outbreak versus outbreak populations), adult weevil density treatment (3 pairs versus 15 pairs), life stage observed, and shoot type. There were significant differences between the proportions of each life stage present within shoots ($F_{2,314}=54.988$, $P<0.001$). Additionally, significant interactions between life stage/adult density ($F_{1,314}=9.890$, $P<0.001$) and life stage/shoot type ($F_{2,314}=4.831$, $P=0.009$) were observed. The implications of these interactions are interpreted in Figure 4.7 using Tukey HSD post-hoc comparisons across offspring stage versus adult weevil density (Fig. 4.7a) and across offspring stage versus shoot type (Fig. 4.7b). No other factors or interactions were significant. Figure 4.7a shows that, despite weevils having a three-month period for development, a significant majority of *M. janthinus* offspring found in high-density shoots were pupae (51%), as opposed to equivalent numbers of pupae and adults in low adult density treatments. There were significantly lower mean proportions of adults within the crowded treatment shoots than within the lower parent density treatments (29% versus 44%). Similarly, within the non-preferred vegetative shoots, a larger mean proportion of offspring were pupae than adults, as opposed to equal numbers of each within preferred reproductive shoots (Fig. 4.7b).

An ANOVA model ($r^2=0.330$, n=902 developed adults) tested four factors as influences on weevil size: weevil gender, larval crowding history of parent weevils, parent density treatment and shoot type. Similar to Chapter 3, male weevils ($\mu=2.73\pm0.98$ mg) were, on average, smaller than females ($\mu=3.14\pm1.25$ mg) and weevil gender was a significant influence on size within the full ANOVA model ($F_{1,886}=28.736$, $P<0.001$). Adult density

treatment accounted for 24% of the size variation ($F_{1,886}$=282.628, P<0.001), whereas the type of shoot used during development was responsible for only 2% of the differences in weevil size ($F_{1,886}$=17.448, P<0.001). As above, the larval crowding experienced by parent weevils did not influence the size of offspring adults ($F_{1,886}$=0.028, P=0.867). The three-way interaction between shoot type, adult density and parent larval history was also significant ($F_{1,886}$=9.205, P=0.002). The interaction plot for these factors (Fig. 4.8) indicates that the size of offspring *M. janthinus* was consistently larger in shoots from the uncrowded adult treatments. Alternating patterns of weevil size occur when parental crowding history (i.e. outbreak versus non-outbreak sites from which adults were collected) and oviposition into preferred or non-preferred shoots are included in the model (Fig. 4.8). A significant, negative correlation was found between oviposition density and mass of adult offspring (r_s=-0.436, P<0.001) when all treatment combinations were pooled (Fig. 4.6b).

Discussion

Field surveys of *M. janthinus* shoot choice and larval survival

Toadflax shoot density and morphology can be highly variable in field settings. Reproductive, or flowering shoots, appear to be much more obvious within toadflax patches near the end of the oviposition period than tall vegetative shoots, with an average of 3.4 times more total linear shoot length (Table 4.1) due to branching. These apparency estimates of shoots, sampled in late-June, are inflated compared to the amount of shoot

material actually encountered by female weevils during the peak oviposition period. They do, however, illustrate the differences in growth morphologies between reproductive and vegetative shoots. A supplementary study of shoot phenology throughout the duration of *M. janthinus* oviposition would be required to determine if reproductive shoots are consistently more apparent to gravid females and whether this factor influences host selection. It is clear that reproductive shoots were much less available to ovipositing weevils within sampled toadflax stands than vegetative shoots, representing a maximum of 14% of the total shoot abundance at sites with low weevil densities (Table 4.2). Early season adult weevil herbivory on toadflax shoot tips further decreased the number of shoots that could have developed into reproductives (Chapter 2), reducing the representation of that shoot type to 3.2% of all shoots at heavily attacked sites. As a result, ovipositing weevils would be expected to encounter vegetative shoots more frequently than the robust reproductive shoots at both outbreak and non-outbreak sites.

Such variations in the density of different toadflax shoot types potentially affect *M. janthinus* oviposition choices. It was observed that the incidence of weevil attack on reproductive shoots was greater than if these shoots were encountered randomly, suggesting that there is host selection by ovipositing females for this robust type of shoot. At sites where weevils have reached outbreak population densities, virtually 100% of available shoots are attacked. It is at pre-outbreak sites that we can observe females electing to oviposit into the larger reproductive shoots.

The patterns of intensity of *M. janthinus* adult attack on toadflax shoots were not identical to those for the incidence of attack. A comparison of the intensity of weevil attack across shoot and site types (Fig. 4.1) showed that ovipositing weevils most heavily used tall vegetative shoots. This corresponds to findings by Pires *et al.* (2000) of non-random attack of a Cynipid galler on larger host shoots and is consistent with Price's (1991) plant vigour hypothesis. Oviposition into reproductive shoots at both non-outbreak and outbreak sites, however, was less intense than within tall vegetative shoots (Fig. 4.1). These results may be explained by a higher growth rate for reproductive shoots between the peak oviposition period and sample date, thus reducing the density of weevils per unit shoot length. Although the hypothesis of different shoot growth rates across shoot types remains to be formally tested, it was shown in the greenhouse oviposition preference-offspring performance experiment that reproductive shoots achieved twice the linear length of vegetative shoots within the same growth period.

At non-outbreak and outbreak sites, a greater proportion of developing larvae in taller, vigorous shoots (i.e. tall vegetative and reproductive) were discovered alive during the mid-season census than in short vegetative shoots. Model fit for this ANOVA (r^2=0.112) indicates that adult population density and the type of shoots used for development did not adequately account for all of the variation in early instar *M. janthinus* survival. This may be an artifact of the low larval populations within shoots at non-outbreak sites (see Fig. 4.2). Although the use of life table analyses, as described in Royama (1981), would provide more detailed stage-specific survival data, this single sample of larvae within shoots detected important offspring performance trends. Despite the capacity of the

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sampled vegetative shoots to support *M. janthinus* development (e.g. Figure 4.1 shows that basal shoot diameters were at least 0.9mm for this shoot type, as required for larval development; Jeanneret and Schroeder, 1992), there was reduced success of weevils developing within short vegetative shoots at all sites. The high mortality of larvae within these small stems, magnified by the avoidance behaviour of female *M. janthinus* toward less acceptable host types, reduces the size of *M. janthinus* populations on this non-preferred shoot type. This type of 'behaviour amplification' is described in detail by Preszler and Price (1988).

The other interesting inference that can be drawn from the larval survival data is that there is no obvious density-dependence during the early instar larval stages. Patterns of mid-season larval survival in the preferred shoots at low weevil population densities are barely significantly different from weevil populations experiencing outbreak levels (Fig. 4.2). Within a limiting resource such as individual toadflax shoots, one would expect to see the effects of intraspecific competition on larval survival at higher offspring densities, as has been found with other endophagous insects (Branson and Sutter, 1985). The field survey data suggest that competition may not be a density-regulating factor early in the development of *M. janthinus*. Consequently, the study of early larval survival is not particularly useful as a measure of overall M. janthinus performance.

Testing the oviposition preference – offspring performance relationship

The greenhouse preference-performance experiment was designed to elucidate whether

host choice patterns observed in the field were consistent with *M. janthinus* oviposition

preferences determined in a controlled setting, as well as to examine how weevil

crowding at different life stages affected these choices and overall success of offspring.

The insects were strictly offered a choice between vegetative and reproductive shoots.

Due to the pattern of toadflax growth in pots, it was not possible to introduce the tall

vegetative shoot category into the experiment to distinguish whether observed

preferences were a result of plant vigour or inherent quality of one shoot type over

another. Individual reproductive shoots were the more vigorous shoots, averaging twice

the growth rate of the vegetatives during the experiment.

The results from the 2x2 factorial experiment confirmed that the host use patterns seen at

field sites were a result of female weevils' oviposition preferences (Fig. 4.4); large,

reproductive shoots received almost twice as many eggs as vegetative shoots. These host

shoot preferences remained stable across different levels of adult crowding during

oviposition and larval crowding histories of parents.

An unique aspect of this study showed that the parent weevils' larval crowding

experience had no significant effect on host use patterns. There are very few studies that

compare host selection by female insects as a function of their larval experience. The

available literature is limited to evaluating host shift and range expansion hypotheses by

testing oviposition choices between insects from different host species (Camara,1997; Craig *et al.*, 1997). Results from these papers commonly show higher levels of offspring performance on host species from which adults were collected. Via's (1986) study exploring the genetic link between oviposition preference and offspring performance in the herbivore *Liriomyza sativae* found that despite the two different host species from which flies were sampled, preferences for one host were consistently expressed. Offspring performance of *M. janthinus*, as measured by survival to adulthood, was similarly not influenced by their parents' crowding experience.

Offspring performance, measured as the proportion of offspring surviving to adulthood, was significantly higher within preferred toadflax shoots (Fig. 4.5). This may have been related to a higher development rate of weevils within reproductive shoots (Fig. 4.7b) or delayed oviposition into non-preferred shoots. Mechanistic hypotheses can be formulated from the life stage patterns in Figure 4.7a. As weevil populations become crowded, larval development may slow compared to non-crowded situations, resulting in fewer offspring reaching the adult stage during the development period. This type of density-dependent regulation within crowded larval populations is often a direct response to declining host quality and/or quantity and is commonly reported in the literature (McClure, 1991). Alternatively, ovipositing females may have been re-priorizing their host choices as space within preferred shoots became limiting, resulting in a shift in oviposition from preferred to non-preferred host shoots as crowding within preferred shoots increased. Delayed oviposition into less preferred shoots would potentially result in higher proportions younger life stages of *M. janthinus* at the time of shoot dissection. A similar functional

response to changing target plant availability has been documented in the herbivorous Chrysomelid beetle, *Zygogramma suturalis* (Reznik, 1993) and in the shoot-galling sawfly, *Euura lasiolepis* (Craig *et al.*, 1989).

Reduction in the size of endophagous insects is generally an outcome of the level of crowding experienced by individual insects as immatures (Black and Krafsur, 1986). *M. janthinus* is no exception, although males tend to be more severely affected by larval density (Chapter 3). The 3-way interaction between source weevil population, adult density treatment and shoot type makes it difficult to interpret the size differences among treatment combinations. It appears, however, in Figure 4.8, that the size of adult offspring is enhanced by development within preferred shoots when populations are uncrowded (3 pair treatments), but is ultimately compromised in a density-dependent manner once the overall weevil populations reach a peak level (15 pair treatments). This interpretation corresponds to results in Chapter 3 regarding the crowding of *M. janthinus* larvae within toadflax shoots and resultant decrease in adult size. Figure 4.6b illustrates the relationship between reduced adult size with increasing density within shoots.

Conclusions

Consistent with the plant vigour hypothesis (Price, 1991), *M. janthinus* exhibits a distinct preference for tall, probably more vigorously growing shoots, both in the laboratory experiment and field study. In the controlled experiment, *M. janthinus* displayed a tight association between oviposition preference and offspring performance. This was

particularly evident in the relationship between host choices made during the experiment and survival to adulthood. Optimal foraging theory states that in endophagous species, such as *M. janthinus*, where larval mobility is restricted, selection favours insects that maximize their reproductive success by choosing the optimum resources for offspring development (Koricheva and Haukioja, 1994). Similar correspondence between host selection by oviposition into vigorously growing shoots, or shoot modules, and optimal larval performance has been documented for other endophagous insects (Carr *et al.*, 1998; Craig *et al.*,1989; Pires *et al.*, 2000).

Resource availability ultimately appears to be the limiting factor in the performance of developing weevils only under high population densities (e.g. Fig. 4.8). Strong *et al.* (1984) hypothesize that intraspecific interactions have little effect on a species' population dynamics, but Auerbach *et al.* (1995) found that leafminer populations were seriously affected by intraspecific competition during the eruptive phase of their dynamics. At peak densities of *M. janthinus*, crowding effects imposing negative feedback regulation of the population may reduce the success of offspring developing within preferred shoots. However, as it only operates at high insect densities, density-dependent larval competition is not sufficient in preventing outbreak dynamics (Eber *et al.*, 2001).

Dodge and Price (1991) found that by comparing the preference-performance relationships of latent and eruptive species, latent species were largely constrained by the shortage of preferred shoots within host patches. This rarity of preferred resources is

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believed to keep herbivore population fluctuations low (Levya *et al.*, 2000). However, this study has shown that *M. janthinus* is not as confined to one rare, preferred host type as some other endophagous insects. The weevils showed a distinct preference in the field study by attacking reproductive shoots more frequently than expected, but also laid high densities of successful offspring in tall vegetative shoots, a shoot type that is still available even at sites with heavy impact on the host plant. The results of the field survey suggest that this flexibility within the oviposition preference hierarchy of *M. janthinus* allows weevil populations to maximize offspring fitness and maintain a strong correspondence between oviposition preference and larval performance, similar to findings by Carr *et al.* (1998). Craig *et al.* (1989) also demonstrated through manipulative experiments that the oviposition preference hierarchy of the shoot-galling sawfly, *Euura lasiolepis*, changed with the availability of preferred resources. The tight association between ovipositon preference and offspring performance on vigorous shoots, combined with the range of acceptable host shoot morphologies and delayed density-dependent population regulation, allows this biocontrol agent to experience eruptive population dynamics in spatially and temporally variable toadflax patches.

This study suggests that biocontrol researchers should be evaluating the population dynamics of individual insect species, instead of accepting the general assumption that all specialist herbivores have latent population dynamics. Rather than species being categorized as latent or eruptive in the strictest sense, there may well be a continuum of population dynamics that are expressed under variable resources. Price's (2000) statement that endophagous specialist herbivores, such as *M. janthinus*, are tightly

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constrained by a shortage of high quality food, resulting in latent species dynamics has

been challenged. The flexibility in the oviposition hierarchy of *M. janthinus* enables this

species to maximize its fitness, and consequently population size, under changing host

conditions. Tests of the relationships between host choice, host availability and offspring

performance may provide the key to determining which proposed biocontrol agents have

the potential to reach outbreak levels in their new environments.

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TABLE 4.1. Characteristics of Dalmatian toadflax shoot types selected for field quantification of *M. janthinus* oviposition preferences. Data are pooled across 12 study sites. Ten shoots of each type were collected and dissected per site (i.e. n=120 shoots per type).

Shoot Type	Primary Stem Length (cm) Mean±SD	Basal Diam. (mm) Mean±SD	# Lateral Branches Median	Range	Total Linear Length (cm) Mean±SD
short vegetative	15.5 ± 4.8	2.0 ± 0.1	0	0 – 4	16.2 ± 4.4
tall vegetative	40.9 ± 8.0	4.1 ± 0.1	0	0 - 14	45.7 ± 15.5
reproductive	68.1 ± 15.3	5.8 ± 0.1	6	0 - 28	155.2 ± 74.6

TABLE 4.2. The availabilities of Dalmatian toadflax shoot types, calculated by multiplying the mean primary shoot length of representative samples of each type (vegetative, n=120; reproductive, n=60 per site category) by the frequency of each shoot type within toadflax stands (Chapter 2). Mean primary shoot lengths across 6 sites per category are presented ± 1 SD.

Site Category	Shoot Type	Mean 1^0 Shoot Length (cm) ± SD	Frequency of Shoots	Index of Availability
Non-outbreak	vegetative	35.7 ± 20.6	0.858	30.6
	reproductive	153.0 ± 66.7	0.142	21.7
Outbreak	vegetative	27.3 ± 15.3	0.968	26.4
	reproductive	157.5 ± 82.3	0.032	5.0
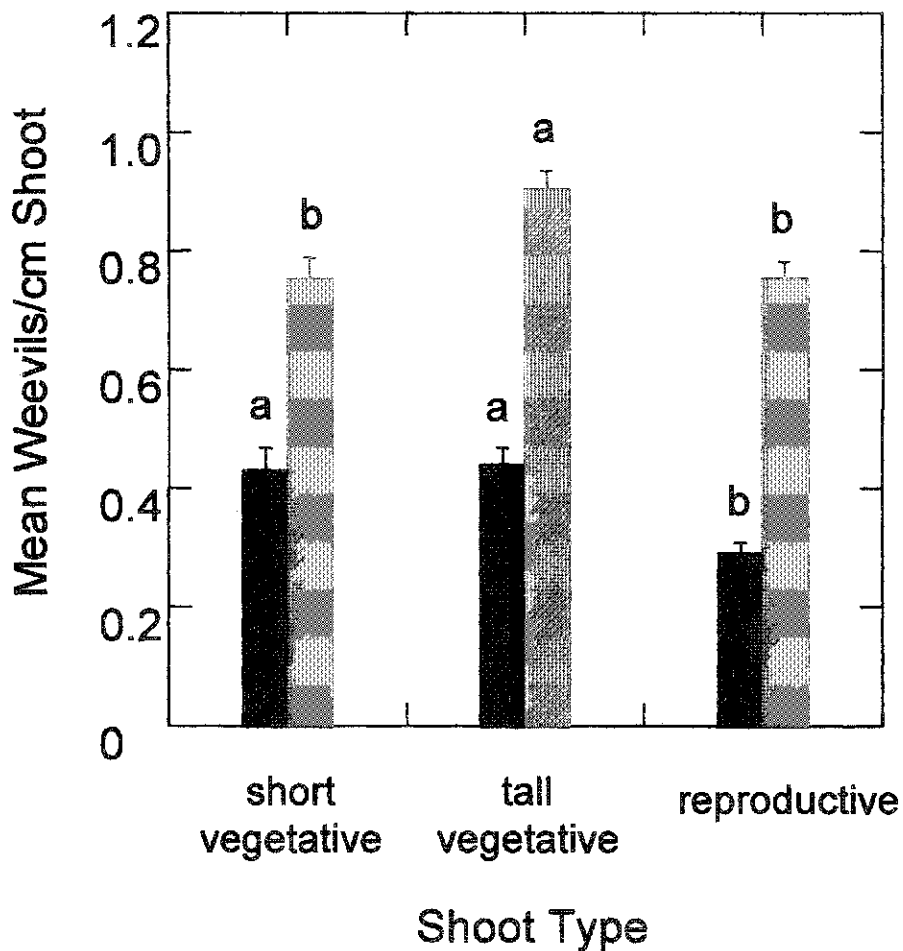
FIGURE 4.1. Attack densities by ovipositing females at non-outbreak sites (■) and outbreak sites (▨) across shoot types. Mean *M. janthinus* eggs laid per cm shoot ± SE are presented, n=60 shoots per site and shoot type. Different letters above bars of the same site type indicate significant differences in egg densities at a 95% confidence level.
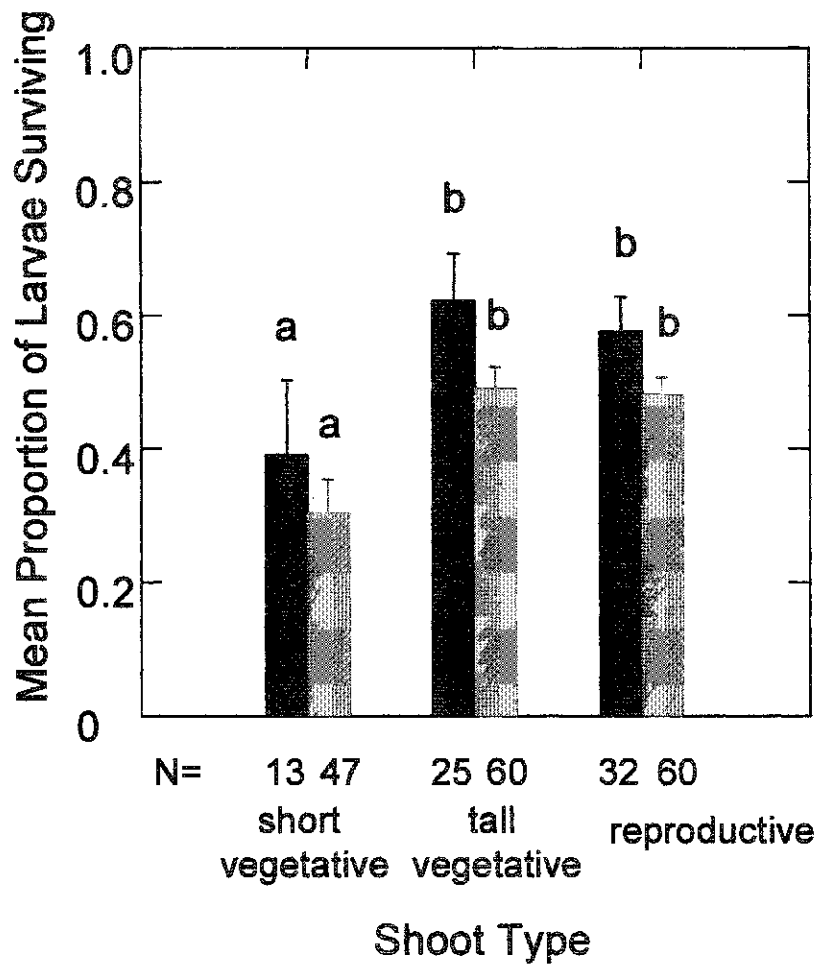
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FIGURE 4.2. Effects of shoot type and site category (non-outbreak ■ , outbreak ▨) on the proportions of *M. janthinus* larvae surviving within toadflax shoots at the end of July 2001. Mean proportions of larvae surviving ± SE are presented. The number of larvae found in shoots of each sample group is listed under the x-axis. Different letters above bars of the same site type indicate significantly different proportions of larval survival at a 95% confidence level.
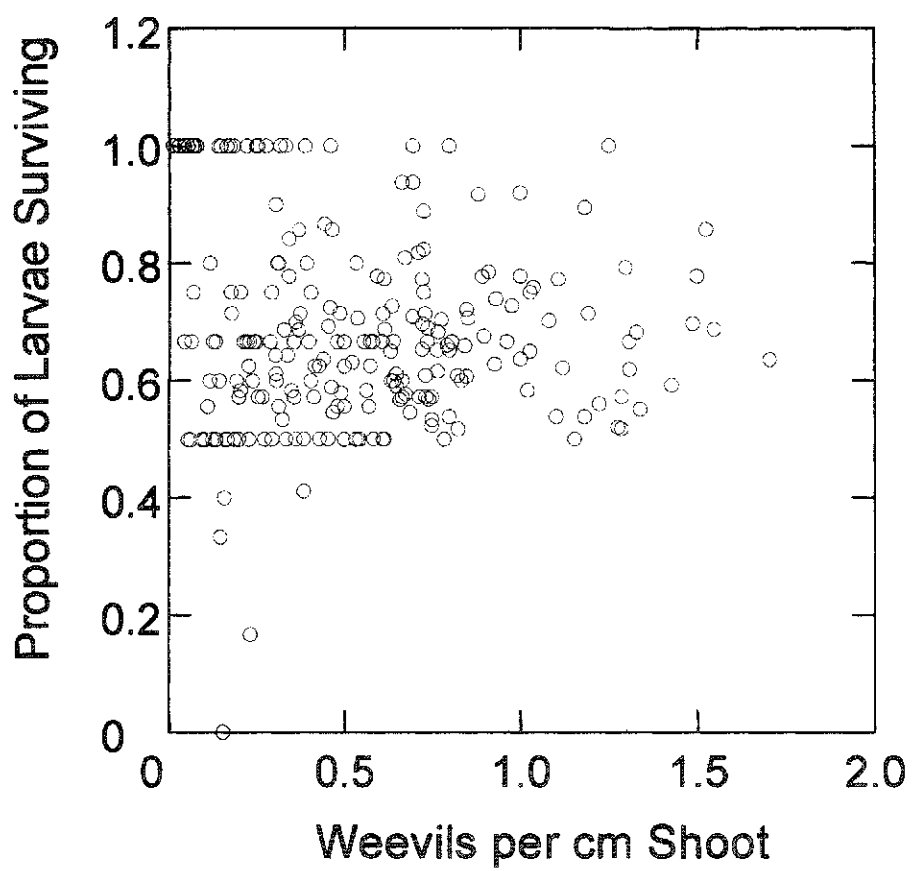
FIGURE 4.3. The association between *M. janthinus* oviposition density and the proportion of larvae surviving within shoots during mid-season dissection (r_s=0.123, n=234 larvae, P=0.060).
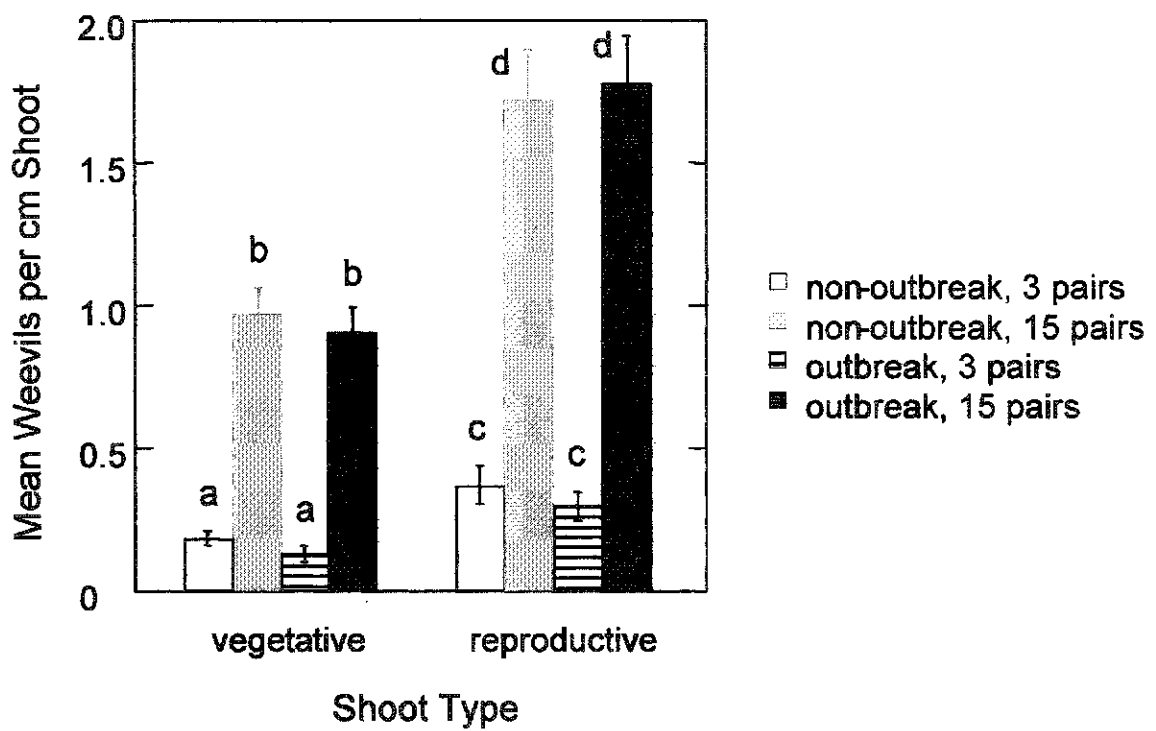
FIGURE 4.4. The influences of adult weevil density, adult weevil source population and shoot type on *M. janthinus* oviposition site selection. Mean oviposition density ± SE are presented, each treatment had 14 replications/shoot type. Different letters above treatments indicate significant differences in egg densities at a 95% confidence level.
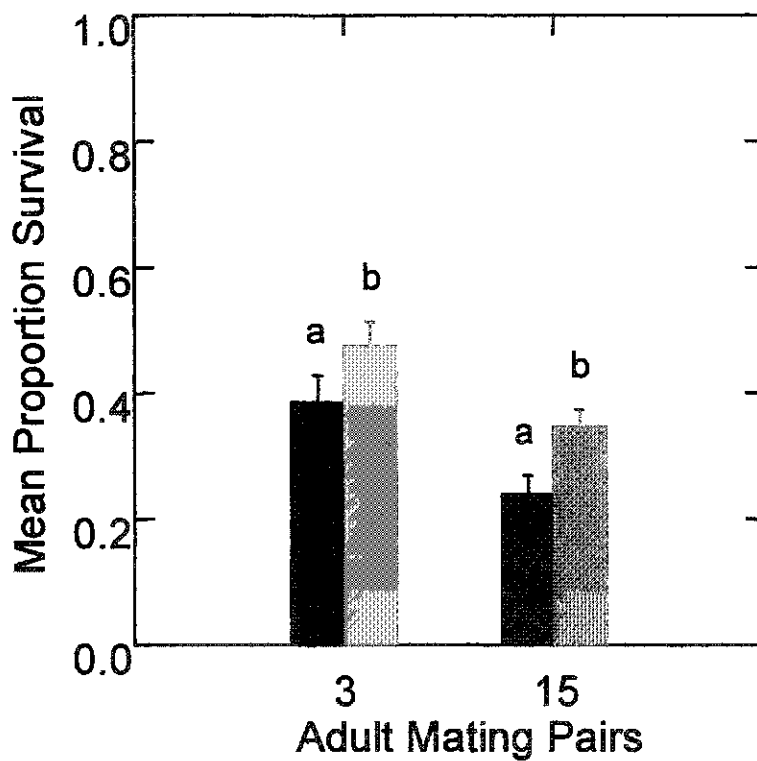
FIGURE 4.5. The effects of adult weevil density and oviposition shoot type choice (vegetative ■, reproductive ▩) on the resulting survival of offspring to the adult stage. Mean proportions of weevils surviving to adulthood ± SE are presented, n=56 replicate toadflax plants balanced across treatment. Different letters above bars of the same density treatment indicate significant differences in survival at the 95% confidence level.
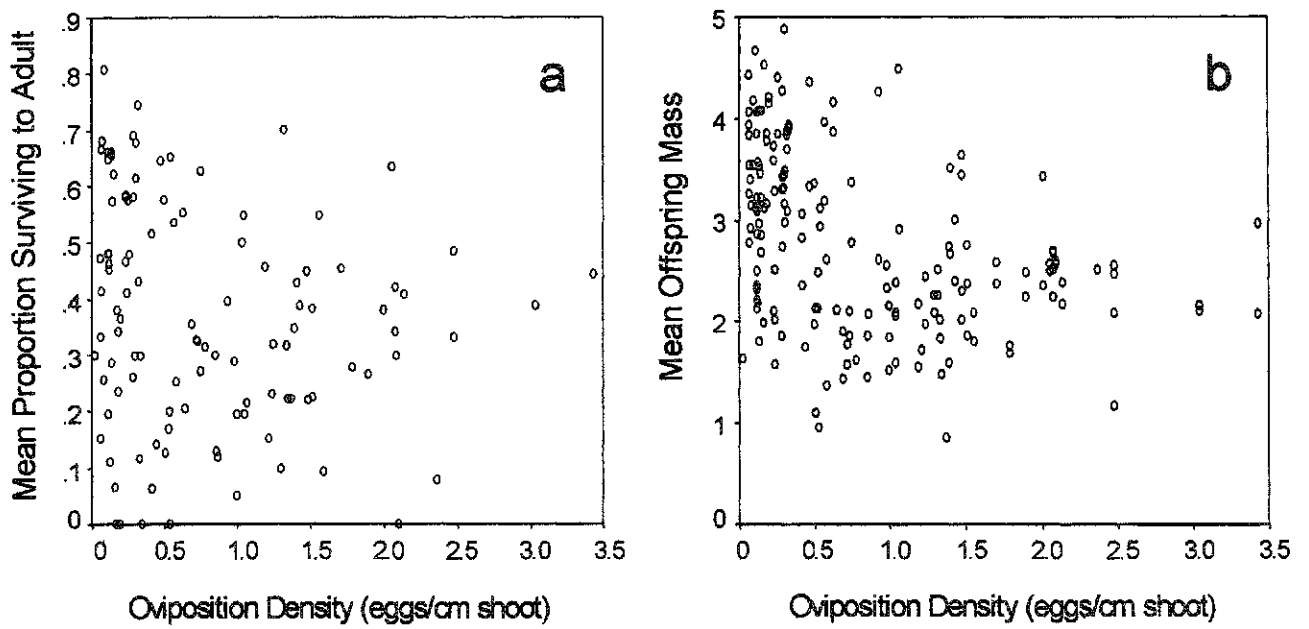
FIGURE 4.6. Associations between the density of adult *M. janthinus* offspring and (a) the mean proportion of offspring surviving to the adult stage (n=112, r_s=-0.186, *P*=0.050), and (b) the adult mean mass (n=175, r_s=-0.436, *P*<0.001).
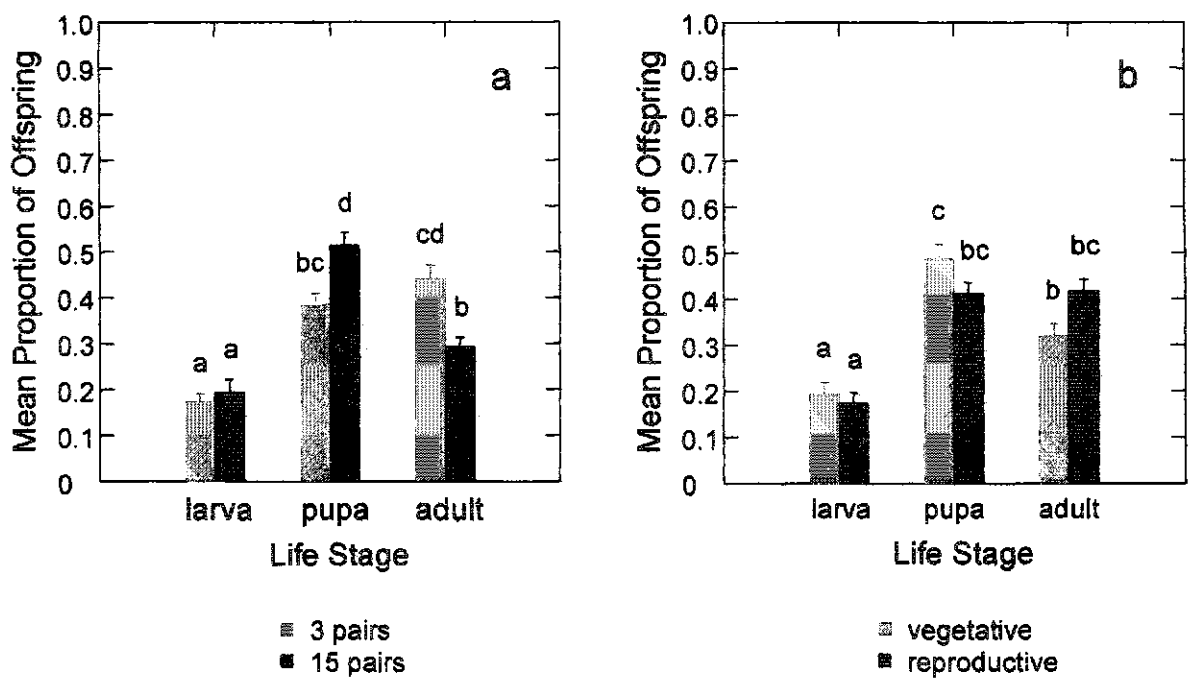
FIGURE 4.7. Summary of the mean proportions of individual life stages found (a) across adult weevil density treatments, and (b) across shoot types. Means ± SE are presented, n=14 per density/shoot type category. Different letters above treatments indicate significant differences in weevil proportions at the 95% confidence level.
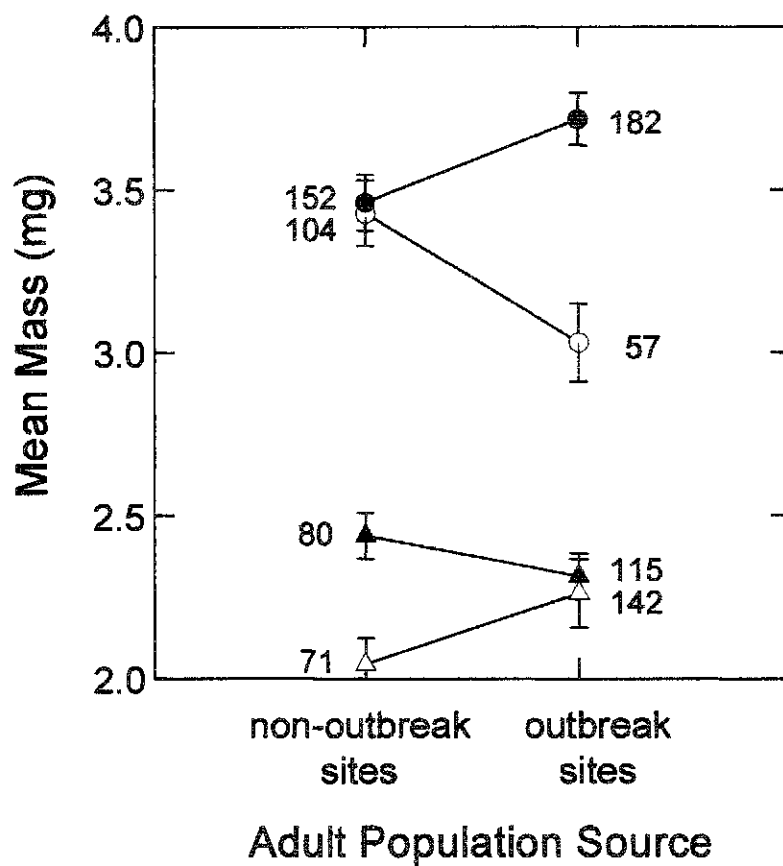
FIGURE 4.8. Interaction plot for parent source population, parent density treatment and shoot type as factors affecting the size of adult *M. janthinus* offspring. Mean adult mass ± SE are presented, sample sizes (n) are variable for each treatment combination and are listed beside their respective symbols. Treatment combinations are represented as: 3 pairs of adults, vegetative shoots (O), 3 pairs of adults, reproductive shoots (●), 15 pairs of adults, vegetative shoots (△), 15 pairs of adults, reproductive shoots (▲).

CHAPTER 5

General Conclusions

Checklists of biological and ecological attributes used to predict potential insect

biocontrol agent efficacy (Goeden, 1983; Harris, 1973; Wapshere 1989) are limited in

their usefulness (McEvoy and Coombs, 1999). However, evaluation of the population

dynamics and interactions of introduced agents with their hosts in new environments is

likely key to predicting whether insects can successfully obtain control over target weeds

(De Clerck-Floate and Bourchier, 2000). McFadyen (1998) warns that determining the

success rates of biocontrol insects requires 10-20 years of agent establishment prior to

analysis. Once established in their new environments, successful insect biological control

agents have several important population attributes in common. These characteristics are

detailed in Harley and Forno (1992) and Wapshere (1989). Several of the key qualities

that allow *M. janthinus* to be a potentially successful agent are discussed below.

Of primary importance to the success of a biological control agent is its ability to

"develop and maintain damaging populations under the ecoclimatic conditions of the

region in which they are to be used" (Harley and Forno, 1992). This study showed that, at

study sites in the West Kootenay region of BC, *M. janthinus* populations rapidly and

reliably built up to outbreak population levels, with greater than a 40-fold increase in

adult weevil abundance within 4 years of release. Crawley (1986*a,b*) found a correlation

between the success of introduced weed biocontrol agents and a high intrinsic rate of

increase.

Although it is too early in the release history of *M. janthinus* to conclusively state that this agent is successful in maintaining high and damaging population levels long term, studying its response to declining host resources reveals its potential to inflict continuing pressure on toadflax populations. Evaluating the density-dependent response of *M. janthinus* to reduced host plant availability showed that weevil populations adapt to crowding by producing smaller individual insects and shifting population sex ratios. Overall fecundity and reproductive success of smaller *M. janthinus* individuals were not affected (Table 3.5), although survival in densely crowded toadflax shoots was somewhat reduced (Fig. 4.5). Intraspecific competition between larval *M. janthinus*, if high, should have been sufficient to suppress weevil populations (Harley and Forno, 1992). This was not observed between 4-8 years after weevil release (Fig. 3.1), suggesting that *M. janthinus* had the potential to achieve substantially large, enduring populations under variable resource availability.

The flexibility within the oviposition preference hierarchy of *M. janthinus* across the range of available toadflax shoots, and the tight correspondence between host choice and offspring survival demonstrated the potential of this agent to persist in a spatially and temporally changing environment. Few endophagous insects have been documented to shift their oviposition behaviour as their most preferred hosts become saturated (Carr *et al.*, 1998; Craig *et al.*, 1989). This perhaps provides a partial explanation as to why many highly host-specific insects, particularly those with limited offspring mobility, have failed as biocontrol agents. Price (2000) describes the 'paradox for biocontrol practitioners' as

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the desire to produce outbreaks in populations of introduced biocontrol agents while employing insects that do not normally achieve outbreak levels in their natural environment due their requirement for rare, high quality host material. The capacity of insects such as *M. janthinus* to accept a range of potential oviposition sites within a host species without compromising offspring performance is a highly desirable trait in selecting potentially successful biocontrol agents.

The population dynamics of a biological control agent are irrelevant if 'critical damage' (Harley and Forno, 1992) to its target host populations cannot be demonstrated. Adult feeding of *M. janthinus* destroyed shoot apical meristems, virtually eliminating flowering and consequently, seed production (Figs. 2.2, 2.5; Table 2.2). The significance of this type of attack is reduced sexual reproductive output of toadflax populations. This study also documented a clear reduction in the aboveground production of toadflax plants within study seasons (Fig. 2.5; Table 2.2). The timing and type of damage inflicted by *M. janthinus* on its host restricts the amount of compensatory regrowth by Dalmatian toadflax plants. Seedpod production from selected plants declined from 2001 to 2002, corresponding with the combined attack of adults and larval *M. janthinus* (Table 2.2). Lawton (1985) describes this type of 'persistent, marked reduction' in a target pest population as the successful hallmark of biological control.

Results from this and other studies (De Clerck-Floate and Miller, 2002; Jeanneret and Schroeder, 1992; Saner *et al.*, 1994) demonstrated that *M. janthinus* has excellent potential as a biocontrol agent against Dalmatian toadflax, at least within the Southern

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interior of British Columbia and similar ecoclimatic conditions. In addition to meeting

many of the criteria outlined by Wapshere (1989) and summarized by Harley and Forno

(1992), the establishment and damage potential of this agent have proven to follow clear

patterns at selected study sites. The major contribution of this study to the biological

control of *L. dalmatica* is to have determined the predictability of this agent throughout

its history of establishment. Although there are many other facets of this agent's

population dynamics that remain to be evaluated before an overall population model can

be constructed, this study provided basic ecological and behavioural data on the

interactions between *M. janthinus* and *L. dalmatica*. This information may be valuable in

the development of prescriptions for use of the agent in BC.

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APPENDIX 1*

Identification of external characters for differentiating between the sexes of adult

Mecinus janthinus **Germar (Coleoptera: Curculionidae)**

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Abstract

Three distinct, secondary sexual characters are described for use in differentiating

between male and female adults of the European weevil *Mecinus janthinus*, introduced to

Canada for biocontrol of the weed Dalmatian toadflax. All diagnostic morphological

characters are external, and two are suitable for sexing live adults under a stereoscopic

microscope.

Introduction

The stem-boring weevil, *Mecinus janthinus* Germar, was introduced from Europe to

Canada in 1991 to control the noxious weeds, Dalmatian and yellow toadflax (*Linaria*

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dalmatica (L.) Mill. and *L. vulgaris* (L.) Mill.)(De Clerck-Floate and Harris, 2002; McClay and De Clerck-Floate, 2002). Since its initial release, *M. janthinus* has established well on Dalmatian toadflax in British Columbia and, to a lesser extent, in Southern Alberta (De Clerck-Floate and Miller, 2002), and is beginning to show promise as a successful weed control agent (De Clerck-Floate and Harris, 2002).

The biology of *M. janthinus* has been described by Jeanneret and Schroeder (1992) for Europe, and by De Clerck-Floate and Miller (2002) for BC. Both adults and larvae of this univoltine species feed on toadflax; the larvae mine and develop to adulthood within growing shoots.

As part of ongoing population and ecological studies of *M. janthinus*, it became necessary to search for external morphological characters that would allow reliable differentiation of the sexes of live adult weevils. Such characters also may be of use to operational staff involved in field collection and application of this biocontrol agents for Dalmatian toadflax management. Described here are three useful morphological characters for rapidly distinguishing adult female and male weevils, two of which can be inspected without injury to live weevils.

Methods

The weevils used in this evaluation were first generation progeny of adults collected in May 2002 from Dalmatian toadflax stalks near Grand Forks, BC. The beetles were reared

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on potted toadflax under greenhouse conditions and removed from toadflax stalks as adults in October 2002.

A total of 285 weevils were recovered from the greenhouse-reared plants and chilled at 5°C for 24-48 hours prior to being examined under a Leica MZ9.5 dissecting microscope. Two of the external characters, present only on males, involve leg morphology and were easily distinguished on live, chilled adults under 10-30x magnification. Individual weevils were marked as suspected males or females based on these two external features and preserved in 70% ethanol for 24 hours. A third external morphological difference between the sexes was evident in the structure of the pygidium. Examination of the pygidium was performed on the preserved weevils after removal of the elytra. Subsequently, abdominal dissections were performed and the internal genitalia of each weevil were used to confirm external sex features.

Images obtained with a scanning electron microscope (SEM) images are presented to illustrate the external features identified as secondary sexual characters. Preparation for SEM included immersion of live adult weevils in liquid nitrogen. Legs and abdomens were mounted on metal stubs (1.2 cm diameter) with M-glue (Agar Aids). Specimens were sputter-coated with gold using a Denton Vacuum Desk II and were observed using a Hitachi S-570 scanning electron microscope at 7.0 kV. Images were digitally captured using Quartz PCI 4.0 and were processed using Adobe Photoshop 7.0.

Results and Discussion

The sex ratio of this greenhouse-reared population approximated 1:1, with 148 females and 137 males. This is consistent with observations made from weevil populations near Grand Forks, BC, although some field populations deviated to a 1.5:1 ratio (Carney and De Clerck-Floate, unpublished).

As in dissections of *Cylindrocopturus adspersus* (Reinecke, 1981), the spiculum ventrale of female weevils is well sclerotized and melanized for rapid distinction from male weevils. Presence or absence of this internal morphological feature was used to determine the gender of dissected weevils.

Character 1. Presence of Hind Tibial Spur

The first external sexing character examined was the presence of a spur on the distal end of the hind tibia in male weevils (Fig. 1). This tibial spur is greatly reduced or absent in females (Fig. 2). Both sexes have obvious distal tibial spurs on the fore and middle legs; it is only the hind tibia that differs between males and females. Accuracy of differentiating between sexes of live weevils using this character (n=285) was 99%, based on the internal morphology of each weevil. Errors favoured misidentification of males as females, as each of the four incorrectly identified male weevils was very small with poorly developed tibial spurs.

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Character 2. Presence of a Denticle on the Pro-femur

The second external feature proved to be more robust for differentiating the sexes, as it is

larger and, consequently, more visible under a stereoscope. In males, a denticle is present

at the distal end of the pro-femur, where it reaches its widest (Fig. 3). This denticle is

accompanied by crenulations directed toward the femur-tibial joint (Fig. 3). The pro-

femur of the female is enlarged at the same location but smoother, without protuberances

(Fig. 4). Accuracy in using this character for differentiating the sexes of live weevils,

compared to abdominal dissections, was 100%.

The most useful and accurate techniques for differentiating the sexes of *M. janthinus* are

those involving the examination of external leg morphology. Male weevils have

additional structures on both fore and hind legs, presumably to aid in copulation. Both

sexes are adept at climbing, with very hairy tarsi and tibial spines on front and middle

legs. The presence or absence of both hind tibial spurs and pro-femur denticles can be

observed within seconds of placing an adequately chilled weevil under a stereoscopic

microscope. Observation of these two external features provides rapid, accurate means of

sexing large numbers of adult weevils.

Character 3. Morphology of Pygidium

The structure of the pygidium is the third external character that can be used to

differentiate male and female weevils. The pygidium of male weevils is composed of two

articulating plates (Fig. 5), whereas in the female, the pygidium is a single plate of similar

dimensions as the two male plates (Fig. 6). The pygidium is the only highly sclerotized structure on the dorsal side of the abdomen and can be easily identified once the elytra are removed. The sex of each of the 285 preserved weevils was correctly predicted using this character.

The use of pygidium morphology is less useful in differentiating male and female weevils than observing the other secondary sexual characters, although it has proven to be as accurate as using either of the other two external features. The disadvantage to using this character is that, as the elytra naturally cover the abdomen tightly, it is necessary to force them away from the abdomen in order to see the pygidium. It is not possible to observe the pygidium of *M. janthinus* by pulling the last abdominal sternite down with a pinning needle, as is done with *Larinus minutus* (Kashefi, 1993). Damage to live weevils is inevitable with this method, thus the application of this morphological feature for sexing weevils may be limited to preserved specimens.

Acknowledgements

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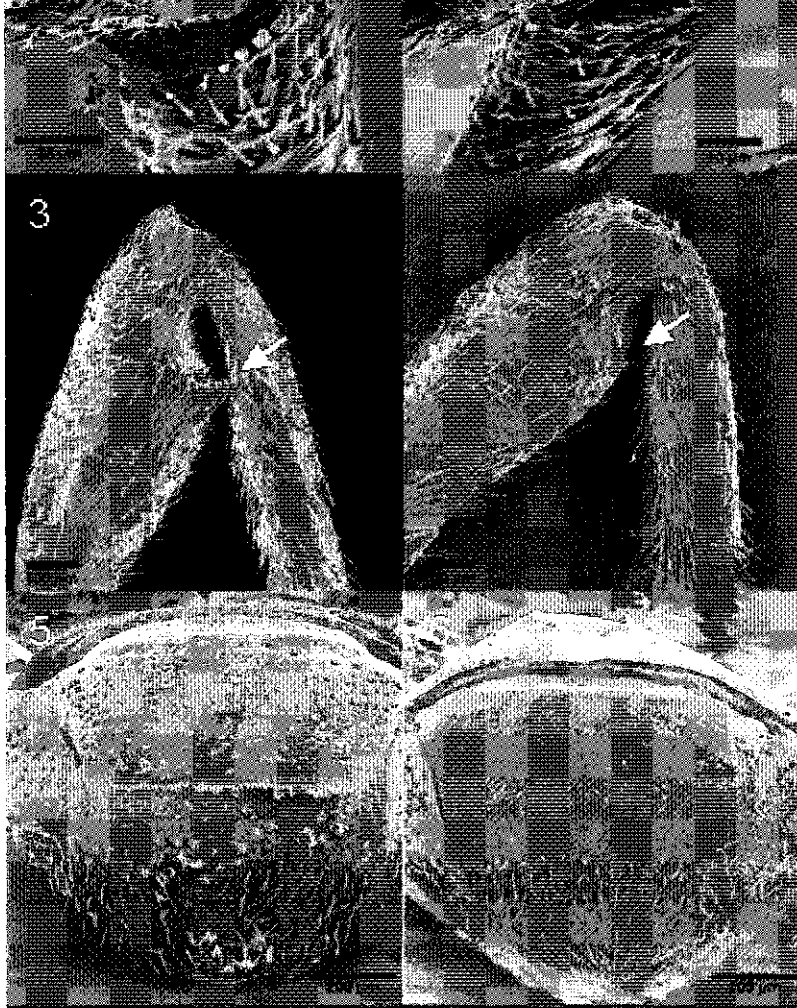
FIGURES 1, 3 and 5. Male *M. janthinus* external morphology. Left hind tibia, with a spur projecting from the distal end opposite the tarsi (Fig. 1). Left pro-femur, with a denticle facing into the body when weevil is standing (Fig. 3). The heavily sclerotized two-plate pygidium (Fig. 5).

FIGURES 2, 4 and 6. Female *M. janthinus* external morphology. Left hind tibia, without a spur (Fig. 2). Left pro-femur, with an enlarged, smooth connection between the femur and tibia (Fig. 4). The single plated pygidium (Fig. 6). Note the similar dimensions of male and female pygidia.

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