

**Ecological Interactions Between Insect Herbivores and Their Host Plant in a Weed
Biocontrol System**

Michael Lewis Crowe

B.Sc., University of Lethbridge, 1999

A Thesis
Submitted to the School of Graduate Studies
Of the University of Lethbridge
in Partial Fulfillment of the Degree

MASTER OF SCIENCE

Lethbridge, Alberta
September 24, 2003

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Abstract

The role of interspecific competition as a regulating force in natural populations has been controversial, especially for phytophagous insect communities. A series of manipulative experiments using enclosure cages were conducted to evaluate the role of interspecific competition between a weevil and a fly, two seed feeding agents released against spotted knapweed in North America. The fly, an inferior biological control agent, was the superior competitor. Consequences of the antagonistic interaction included reduced seed destruction compared to if just the weevil was released on its own. The role of plant phenology on insect herbivore density was also assessed. The implications of phenology-induced variation in insect density were evaluated with respect to competition between the fly and the weevil and were found to be important. Hypotheses of four plant-mediated mechanisms of interspecific competition were also tested. Results support resource preemption as a competitive mechanism.

Acknowledgements

Acknowledgement goes to the BC Ministry of Forests, AAFC Matching Investment Initiative, and to the Canadian Pacific Railway for funding support.

I would like to acknowledge my thesis committee members Dr.'s Ralf Cartar, John Bain, and Drew Rendall. I appreciate your support, insight, and commitment, and for the meaningful contribution each of you have made, a contribution that extends beyond the planning of experiment and the development of logic.

To Stephanie Erb, David Hayman, Andy Jensen, Jeff Rau, Monte Thomson, and Ray Wilson: without each of you I'd still be looking for tent poles, dissecting seed heads, digging trucks out of lakes, vacuuming weevils out of cages, reading Systat manuals.... Thank you for generosity and support. More importantly, thank you for a friendship that's been unconditional, sincere, and something I'll treasure the rest of my life.

To Dr. Rob Bouchier, supervisor extraordinaire, hockey team picker not-quite-there. Thank you for the freedom to pursue a topic that was of interest to me and then for all of the support that has made this research and thesis possible. It was an honor for me to study in your lab and under your direction and tutelage. I was as intellectually stimulated and challenged at the end of my time here as I was at the beginning, which I believe speaks to your ability to connect, foster growth, and facilitate learning. Thank you for allowing me to study with you; it has been one of the more rewarding experiences of my life.

I would like acknowledge my parents, Terry and Julie Crowe. I could never have accomplished something like this without the principles and values you've instilled. Hard work, self-discipline, the importance of education, and the list goes on. Your support to my family and to me over the last couple of years is especially appreciated, your commitment to ensuring I have every opportunity to succeed has never changed. I love you both.

Most of all, to Kathy, Sydney, and Bray. Thank you Kathy for putting your educational dreams on hold and allowing me to pursue one of mine. Your support for everything I've done has always been unconditional and generous. I'm the luckiest guy in the world. By the way Sydney and Bray, Daddy's home from BC now. I love of you guys, and I can't wait for the rest of my life together with you.

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Thesis Introduction

Communities are groups of populations that interact in space and time. A primary research focus of community ecologists has been to identify the processes and mechanisms controlling the dynamics of these interacting populations (Milne 1984, Begon et al. 1996). Population processes generally accepted as being important include predation, parasitism, herbivory, dispersal, and intra- and interspecific competition. Each of these is then affected by a suite of abiotic factors, such as physical disturbance and climatic regimes. Ecologists attempt to describe how each of these factors, on their own and in combination, influence the distribution and abundance of organisms (Menge and Sutherland 1967, Hunter and Price 1992, Turchin 1995).

Invasive species, and invasive plants in particular, pose a serious threat to community structure and ecosystem function worldwide (Williamson 1996, McFadyen 1998, Devine 1999, De Clerck-Floate and Bouchier 2000, Mack et al. 2000, Pearson et al. 2000, Sala et al. 2000, Schaffner 2001, Sanders et al. 2003, Simberloff 2003 and references therein). They devalue rangelands, crops, and forests (Pimentel et al. 2000), threaten native species (Wilcove et al. 1998, McEvoy 2002), reduce biodiversity (Samways et al. 1996), enable additional invasions by providing food and cover, and can hybridize with native species, jeopardizing native gene pools (Randall 1996, Lodge and Shrader-Frechette 2003). Once introduced plants are released from indigenous limiting factors, their populations may experience exponential growth and dominate portions of the landscape (Louda et al. 2003). Most invasions are irreversible (Ewel et al. 1999).

Human activities often disrupt ecosystem function and structure, impairing processes essential to the health of native communities, and provide opportunities for

invasive species to establish (Ewel et al. 1999, Tilman and Lehman 2001, Shea and Chesson 2002). And not only do human activities facilitate biological invasions, but they have also accelerated their rate (Lodge and Shrader-Frechette 2003).

Reducing environmental and economic impacts of invasive plants is one of the most urgent challenges facing land managers and conservation biologists (Allendorf and Lundquist 2003, Shea and Chesson 2002), yet presently, the impacts of invasive species remain ‘under-researched and under-appreciated’ (MacFadyen 1998).

Weed Biocontrol

As the scope and scale of problems associated with the establishment and spread of invasive species continue to increase, so does interest in biological control as an option for managing areas affected by them (Louda et al. 2003). Classical weed biological control is the intentional release of coevolved natural enemies against a target host plant (McEvoy et al. 1991, Muller-Scharer and Schroeder 1993, Murdoch and Briggs 1996, McEvoy 2002). Theoretical advantages of biological control include the institution of a self-perpetuating, long-term, cost effective, and environmentally safe solution to invasive species management (McClure 1983, Muller-Scharer and Schroeder 1993, Heinz and Nelson 1996, Mack et al. 2000, Louda et al. 2003). The objectives are to maintain low population levels of the target pest and to facilitate its replacement with more desirable species (Myers and Harris 1980). The practice of biological control has been referred to as ‘applied population dynamics’ (Murdoch and Briggs 1996).

Debate continues over the merit of two different agent-release strategies commonly employed in weed biocontrol systems (McEvoy et al. 1993, Blossey et al.

1996, Murdoch and Briggs 1996). One approach calls for the identification and screening of all potential agents, but then releasing just the single most effective one, the silver bullet, against the target weed (Myers 1985). The alternative method, the cumulative stress model, involves the release of multiple agents against the target species in an effort to reduce the competitive abilities of the plant (Harris 1985, 1991, Zwolfer 1985).

Although the cumulative stress model is presently the most common approach in weed biocontrol (May and Hassell 1981, Hogarth and Diamond 1984, Kakehashi et al. 1984, Godfray and Waage 1991), the assumption that all established insects will contribute to control of the target weed has been criticized (Myers 1985). Results of multiple species introductions are variable, and although the effects can be additive and successful (see McEvoy and Coombs 1999), there are cases where they have made little difference or have even had negative consequences (Myers 1985).

One such negative consequence is the increased potential for unanticipated ecological risks (Howarth 1991, Simberloff and Stiling 1996, Louda et al. 1997, 1998, Strong 1997, McFadyen 1998, Louda 2000,). As the number of exotic introductions continues to increase, so does the likelihood of non-target effects, even though the risk of such effects is thought to be quite low (McEvoy and Coombs 1999, Ewel 1999). Although non-target effects of weed biocontrol agents have been documented, the risk they pose must be evaluated against the threat the invasive weed poses to ecological processes, as well as the trade-offs associated with other control practices, such as the application of herbicides (Harris 1985).

Associated with an increase in the number of biocontrol agents released against a target pest is an increase in the likelihood that some interactions between agents will be antagonistic. One potential consequence of an antagonistic competitive interaction between biological control agents is the displacement of a superior control agent by a less effective, but competitively superior, agent; leading to a less effective biocontrol program (Ehler and Hall 1982, McEvoy and Coombs 1999, Collier and Hunter 2001). Crawly (1986) noted, however, that competition limited the effectiveness of weed-biocontrol organisms in only 13% of cases and McFadyen (1998) argues that this concept has been disproved altogether.

Interspecific Competition

Determining the role of interspecific competition in community structure has long been a central theme in ecology, a 'popular pastime of community ecologists' (Abrams 1990, see also Stiling 1988, Evans 1989), and occasionally the source of intense debate (Schoener 1982, Karban 1989), particularly as the theory applies to phytophagous insect communities. One view is that competition is fundamentally important to the structure of a community and an overriding ecological process (Faeth 1987, Denno et al. 1995, Stewart 1996, Reiz and Trumble 2002). The opposing view is that interspecific competition is rare, and that when it does occur, has little influence on community structure (McClure and Price 1975, Connell 1980, Stiling 1980, Thornhill 1980, Lawton and Strong 1981, Kareiva 1982, Strong 1982, Shorrocks et al. 1984, Strong et al. 1984a, Jermy 1985, Walter 1988, Begon et al. 1996). This view maintains that other factors, such as responses to environmental variation (Wiens 1977), dispersal, host-plant

dynamics (Lawton 1982, Strong et al. 1984b, Auerbach et al. 1995), and natural enemies (Hairston et al. 1960, Schoener 1974, Connell 1975, Akimoto 1988), often keep insect densities below the level at which competition might occur (Begon et al. 1996), or at least at levels where its importance is less than that of other processes (McClure 1986). Even authors (such as: Connell 1983, Schoener 1983) who argue for a central ecological role for competition in general have questioned the role it plays in phytophagous insect communities (Faeth 1986). The challenge for insect ecologists is to determine the role of competition in 'real world' insect communities (Begon et al. 1996), where it operates in the presence these other factors that reported as being more important (Karevia 1982, Schoener 1983, Evans 1989).

The role of interspecific competition may be more central in structuring phytophagous insect communities used for weed biocontrol. One reason for this view is that weed biocontrol agents are selected based on their host specificity and thus may experience more intense competition compared to other generalist insects, which have the option of switching to an alternative host plant (Karban 1989, Reiz and Trumble 2002). Also, density dependent processes, like interspecific competition, operate with greater frequency, and with greater intensity, as insect populations reach outbreak levels (Bylund and Tenow 1994), a desirable situation for populations of insect biological control agents (Stiling 1988). In their review of interspecific competition, Denno et al. (1995) concluded that competition was more likely to occur between introduced species, and was detected in 91% of the introduced systems they reviewed.

Outbreak events are more likely to occur in the insect populations of biocontrol systems because many of the regulatory factors that suppress insect densities in the native

habitat, such as natural enemies (McGeoch and Chown 1997), are not present in the new community (McClure 1980). Additionally, biocontrol agents are often released into areas where their host plant occurs as a single-species monoculture on the landscape. Strong et al. (1984b) suggest that such a monoculture can often promote population outbreaks for insect species feeding within them, increasing the likelihood that significant competitive interactions will become more important.

Overview

This thesis is divided into two central research themes, each with its own chapter. The objective of Chapter 1 is to investigate the interspecific interactions between two phytophagous insect species introduced as weed biological control agents in a multi-agent system against an extremely invasive weed. The purpose Chapter 2 is to investigate plant-insect interactions between an invasive host plant and its insect herbivore natural enemies and test specific hypotheses of plant mediated competition.

Chapter 1: Interspecific Competition Between Two Weed Biological Control Agents and the Implications for Control of the Target Pest.

Abstract

The role of interspecific competition as a regulating force in natural populations has been controversial, especially for phytophagous insect communities. The implications of competitive interactions between insect herbivores released as biological control agents against an invasive host plant are potentially great. A series of manipulative experiments using enclosure cages were conducted to evaluate the role of interspecific competition between a weevil and a fly, two seed feeding agents released against spotted knapweed in North America. At the seed head scale, the fly, which is an inferior biological control agent, was the superior competitor, as it suppressed weevil attack rates compared to what the weevil achieved when attacking on its own. The weevil did not significantly reduce fly density. Although attack rates were higher when both species were together at a site, the consequence of the antagonistic interaction is that overall seed destruction was not as high as it could have been if the weevil were attacking on its own. The implications for biological control protocol are discussed.

Introduction

Spotted knapweed is an exotic, highly invasive plant that presently threatens the biodiversity associated with native rangeland in southern British Columbia. In North America, the plant is incorrectly referred to as *Centaurea maculosa* Lam, an annual diploid ($2n=18$) species native to central Europe. The North American species is actually the tetraploid ($2n=36$) perennial, *C. biebersteinii* (Harris and Myers 1983, Muller 1989,

Groppe 1990). Harris (1985) identified plant misidentification as one of the major setbacks to weed biocontrol programs and cites spotted knapweed as an example.

Spotted knapweed was introduced into Canada accidentally in 1893 (Watson and Renney 1974). Although the plant is utilized extensively by some native ungulates at certain times of the year (Miller 1990, Groppe 1990), the foliage is unpalatable and fibrous and is generally avoided by livestock and wildlife. Spotted knapweed dominates native vegetation wherever it becomes established and is a potential threat to native plant species. It forms large, dense monocultures, greatly reducing the productivity of valuable native rangeland. Tens of thousands of hectares are presently infested in BC (Duncan 2001), millions in Northwestern US and W. Canada (Story et. al. 2000), with millions more susceptible to invasion (Harris and Cranston 1979). The population expands via peripheral enlargement of existing patch perimeters (Watson and Renney 1974). The rate of spread for spotted knapweed in BC in 1983 was 1000 ha/year (Harris 2003), and newly infested areas are being reported each year (Bourchier et al 2002).

The plant is a short-lived perennial (up to 12 years), annually producing new stalks and seed heads from the root crown (Boggs and Story 1987). It is capable of growing to 145 cm tall and producing over 500 seed heads/plant (pers. obs.). Unattacked spotted knapweed heads produce an average of 19.9 viable seeds (Harris 1980b) and a single plant has an average annual reproductive capacity of 349 (Watson and Renney 1974). Spotted knapweed bolts in early May, and flowers from late June to October with the majority of flowering occurring in July and August (Story et al. 1996, Sheley et al. 1998). Bud development occurs in a basipetal fashion (Little and Jones 1980) and mature seeds are formed by mid-August.

Because of the scale of the problem and the cost associated with conventional treatments (Muir 1986), spotted knapweed was among the first weeds targeted for biocontrol in Canada (Bourchier et. al. 2002).

Urophora affinis Frfld. (Diptera: Tephritidae) was the first insect approved for release in Canada for the biological control of spotted knapweed and was first released in 1970 (Harris 1980b). It has quickly established and is currently the most abundant and widely distributed biocontrol agent on knapweed.

The seed-head fly stimulates the production of a gall at the base of the floral receptacle. The gall functions as a metabolic sink, drawing upon plant resources that would otherwise be utilized in seed production is (Harris 1989b). Hardened galls are also reported to protect the fly from other seed-head insects (Harris 1989). As with many gall formers, the fly has an ovipositional requirement for a seed head of a specific developmental stage, resulting in a limited temporal window in which the fly is able to attack the host (Berube 1980, Harris 1989). Each female lays an average of 120 eggs (Zwolfer 1970) and each individual larvae produces its own gall. Oviposition takes place during the pre-flowering phase of bud development. Berube and Zacharuk (1984) provided evidence for pheromone glands that produce oviposition-detering pheromones.

Each gall reduces seed production by 1-2 seeds (Harris 1980b, Story 1977). Story and Anderson (1978) reported gall densities ranging from 0.3 to 2.25/head. The fly is capable of attacking as many as 99% of the seed heads at a site and can reach average densities of 9.3 galls/head (Story and Nowierski 1984).

The seed head weevil *Larinus minutus* Gyll. (Coleoptera: Curculionidae) was first released in Canada in 1991 to supplement the attack rates and increase the level of seed

reduction already being achieved on spotted knapweed by other biocontrol insects (Bourchier et.al.2002). The weevil is the most recently introduced seed head insect and may be the most promising agent on knapweed (Seastedt et al. 2003).

The biology of the weevil was reviewed by Groppe (1990). On spotted knapweed, a single larva will consume approximately 25% of the seed within a head, but will utilize remaining seed in the construction of a pupal chamber, and therefore is capable of destroying the entire contents of the capitulum. Realized fecundity averages 66 eggs/female and the species achieves an attack rate of 21.3% in its native range. Adults emerge from over-wintering sites in the soil in the early spring and oviposition takes place during the flowering phase of bud development, which occurs in July and August.

The objective of this chapter is to investigate the interspecific interactions between the fly and the weevil to determine: 1) whether or not interspecific competition is occurring, 2) what effect interspecific competition has on the populations of both insect species, and 3) what impact both insect species are having, individually and collectively, on their host plant.

These objectives are realized through a series of manipulative field experiments conducted during the 2001 and 2002 field seasons in the Kootenay region of southern British Columbia. Enclosure cages and insect density manipulations were used to evaluate interspecific interactions between weed biological control insects as well as the impact of those insects on their target host plant.

It was predicted that the weevil would be the superior competitor on account of its tendency to destroy the entire contents of an attacked seed head, which would include

any initiated fly galls, during larval development. It was also predicted that a greater proportion of total seed would be destroyed as the weevil, the superior biocontrol agent, displaces the fly, which is an inferior agent.

Materials and Methods

Wapiti Lake Cage Experiment 2001

The study site was located at Wapiti Lake, near Jaffray, B.C. The vegetative community consists of a mixed-conifer over-story with a native bunch grass community dominating the under-story. Native bunch grasses are high valued forage species and the site is an important winter range for native ungulates and summer range for grazing livestock. An isolated patch (25x35 m) of spotted knapweed occurs along the shore of the lake and was used for this experiment.

Eight enclosure cages (10 ft l x 10 ft w x 7 ft h and covered with a fine mesh screening) were placed into the patch of spotted knapweed. Old seed heads were manually removed from each cage to prevent the existing, over-wintering fly population from emerging into the experimental cages.

Treatments consisted of a control (no insect released), the fly released on its own, the weevil released on its own, and both species released together, and were randomly assigned to the enclosure cages. Adult flies were collected from a nearby population with a sweep net and introduced into the cages between July 11th and 13th, 2001. Adult weevils were introduced on July 14, 2001, prior to the onset of knapweed flowering. Release rates were 400 adults of each species. A 1:1 sex ratio was assumed for populations of both species.

Once the flowering phase of the host plant was complete, approximately mid-September, 100 seed heads from each of the release treatments, and 90 seed heads from the control treatment, were harvested. Each seed head was dissected in the laboratory and larvae within each head were identified and counted. Seed reduction per treatment was estimated by multiplying the number of individuals of each species occupying each seed head by the number of seeds each individual is reported to destroy, and then subtracting that value from the mean number of seeds produced per spotted knapweed seed head as reported in the literature (Woodburn 1996).

Mean number of each insect species per seed head and proportions of seed heads attacked by each species were used as measures of competition. The effect of competition on insect density was analyzed using two nested ANOVA's, one for each species, on rank-transformed mean attack densities (Conover and Iman 1981), with treatment as the fixed factor and enclosure nested within treatment. Expected proportions of attacked spotted knapweed heads, by species, were calculated for the treatment where both species were released together and were based on observed attack rates in the individual species treatments. The expected vs. observed proportions for attack rates and estimated seed reduction were analyzed using goodness of fit tests (Zar 1984). Where indicated, Bonferroni's correction was applied to post hoc interpretations of pairwise comparisons between proportional data analyzed with contingency tables.

Rabbit Ridge Cage Experiment 2002

The study site was located at Rabbit Ridge, near Elko, B.C. The vegetative community consists of a sparse, mixed-conifer over-story with a native bunch grass

community occupying the open under-story. Spotted knapweed presently dominates large areas of the landscape and populations of both the fly and the weevil are established at the site.

Twelve enclosure cages (10 ft l x 10 ft w x 7 ft h and covered with fine mesh screening) were placed into an existing patch of spotted knapweed. Each cage was then divided in half using mesh partitions, producing 24 enclosure cages measuring 5 ft l x 5 ft w x 7 ft h. Old seed heads were manually removed from each cage to prevent overwintering individuals from established fly populations from emerging into the experimental cages.

Four replicates of six treatments were randomly assigned to the enclosures. The treatments consisted of: 1) a control (no insects released), 2) the fly alone at a low-release rate (100 individuals), 3) the fly alone at a high-release rate (300 individuals), 4) the weevil alone (200 individuals), 5) the fly at the low-release rate (100 individuals) with the weevil (200 individuals), and 6) the fly at the high-release rate (300 individuals) with the weevil (200 individuals). Adult flies were collected from a nearby population with a sweep net and introduced into the cages on July 4, 2002, which coincided with the peak flight period for that species. Adult weevils were introduced on July 11, 2002, prior to the onset of the flowering stage of the host plant. Both species were released at a 1:1 sex ratio.

This experiment differed from the 2001 Wapiti Lake cage experiment in one important aspect. This design was a modification of the additive design described by Cousens (1991) and reviewed by Inouye (2001) in which the density of one species, the weevil, was held constant in all treatments while the density of the other, the fly, was

manipulated (Connell 1983). Such a design allows for any significant competitive effects to be detected (Schoener 1983). This design also included treatments where both species were released independently, allowing interspecific competitive effects to be discussed relative to intraspecific competition.

Thirty plants per cage were harvested at the end of August and were brought into the laboratory where the seed heads were dissected. Only heads that had completed the flowering phase were dissected because only seed heads that reached the post-flowering stage of development could have been attacked by both species. Individuals of each insect species were identified and counted.

Because any interaction between the larval stages of these two species would need to occur within a given seed head, competitive effects were measured at the seed head scale. Attack densities for each species were square root transformed ($\sqrt{x+0.5}$) and the competitive relationship between the two species was assessed using two 1-way ANOVA's, one for each species as the response variable and treatment as the factor. The relationship between within-head distributions of flies and weevils were correlated. Where indicated, proportions of heads attacked by each species were analyzed using contingency tables and Pearson's chi-square statistic was used to measure significance at $\alpha=0.025$, accounting for Bonferroni's correction (Zar 1984).

Heinz Cabin Cage Experiment 2002

The study site was located in the Pend d'Oreille valley in southern British Columbia. The site is a primarily open, bunchgrass community with a scattered mixed-conifer over-story. A large, well-established patch of spotted knapweed dominates the

site. Both insect species are present at the site; the fly has likely been there for at least 15 years and the weevil was released in 1998.

Ten transect lines were established along the length of the patch. Transects were spaced five metres apart and were approximately 75 m long. Along each transect, 9 cages (1m x 1.5m) were randomly placed over the individual plants. The cages were made from finely meshed aluminum screen that was sealed on three sides and then stapled to two 36-inch wooden stakes. All old stalks were manually removed from within each cage to prevent over-wintering flies from emerging into the cage.

The fly density on caged plants was manipulated by exposing a randomly selected cohort of ten plants to attack by the fly for a period of one week, and then replacing the cages to prevent any additional attack. Cohorts covered the entire flight period of the fly, which lasted from June 11 to July 31. Prior to the onset of the flowering stage of the host plants, all of the cages were removed and the plants were exposed to attack by the weevil. Additional treatments included: a control, consisting of 10 cages that were in place at the beginning of the season and never removed; a cohort of plants exposed to the weevil only, consisting of 10 cages that were covering plants for the entire flight period of the fly and then removed; as well as 10 uncaged areas that contained plants exposed to attack by both species for the entire season.

At the end of August, when the flowering phase of the host plant was complete, all plants were collected from within the cages and 20 seed heads per cage were dissected. The 20 uppermost seed heads within each cage were selected for dissection to ensure that heads of similar phenological age were being used for between-cage

comparisons. Individuals of each insect species within each head were identified and counted.

Attack densities of the two species within the cages were analyzed with correlations on log-transformed totals. Changes in the mean attack densities of the weevil between plants exposed to attack by the fly and plants from which the fly had been excluded were compared with a t-test. Proportions of heads attacked by each species were analyzed using contingency tables and Pearson's chi-square statistic was used to evaluate significance at $\alpha=0.025$. Expected vs. observed attack rates on uncaged plants were analyzed with goodness-of-fit tests (Zar 1984). The relationship between attack patterns and seed reduction was analyzed with a correlation between arcsin square root transformed proportions of reduced seed and log transformed weevil:fly attack ratios.

CPR Experiment 2002

This study was executed along a 35-mile stretch of railroad right-of-way between Fernie and Elko, British Columbia. Each site was subject to frequent disturbance and occupied habitat between gravel railroad ballast and adjacent forested areas.

Twelve isolated patches (approximately 30-50 m²) of spotted knapweed were selected along the right-of-way and three replicates of four treatments were randomly assigned to the patches. The four treatments consisted of a control (neither species released), the fly released on its own, the weevil released on its own, and both species released together. Prior to spring emergence, all old stalks were manually removed from each of the twelve sites to prevent recruitment from over-wintering individuals from established fly populations. It was assumed that the weevil would not be present, as no

previous releases had been documented in the region where the experiment was conducted. Releases of adult flies (400 individuals per site) were made on July 4, 2002 and the adult weevil releases (400 individuals per site) were made on July 11, 2002.

At the end of August, 20 plants were randomly harvested from each site. Attack rates for both species were calculated by dissection the 10-most terminal heads on each plant to ensure comparisons of phenologically similar heads on different plants.

To determine the effect of release rate for each species on attack densities, the mean number of insects per seed head was compared at release and non-release sites using a t-test.

Results

Interspecific Competition

There were significantly more weevils per seed head when the weevil was released on its own compared to when it was released in combination with the fly at Wapiti Lake, Rabbit Ridge, and Heinz Cabin ($F=58.912$, $d.f.=3,4,382$, $p<0.001$, Fig. 1.1a; $F=3.117$, $d.f.=2,289$, $p=0.046$, Fig. 1.1b; $t=9.724$, $d.f.=488$, $p<0.001$, Fig. 1.1c, respectively). There was no similar effect on the number of flies per seed head when it was released in combination with the weevil at Wapiti Lake, and for both high and low fly release rates at Rabbit Ridge ($F=0.479$, $d.f.=1, 388$, $p=0.489$, Fig. 1.1a; $p=1.000$ Fig. 1.1b, respectively). The negative relationship between the fly and the weevil is plotted in Figure 1.2 ($n=66$, $r=-0.597$, $p=0.003$).

Distributions of both insect species in seed heads from within the combined treatments at both Wapiti Lake and Heinz Cabin were compared to an expected

distribution based on random association. The proportion of seed heads attacked by just the fly was significantly higher than what was expected at both Wapiti Lake and Heinz Cabin ($\chi^2=28.51$, d.f.=3, $p<0.05$, Fig. 1.3a; $\chi^2=55.62$, d.f.=3, $p<0.05$, Fig. 1.3b, respectively). The proportion of seed heads in which both species were attacking was significantly lower than what was expected ($\chi^2=8.45$, d.f.=2, $p<0.05$, Fig. 1.3a; $\chi^2=16.42$, d.f.=2, $p<0.05$, Fig. 1.3b, respectively). There was no significant difference between expected and observed values for the proportion of spotted knapweed seed heads attacked by just the weevil ($\chi^2=2.69$, d.f.=1, $p>0.05$, Fig. 1.3a; $\chi^2=0.55$, d.f.=1, $p>0.05$, Fig. 1.3b, respectively).

The effects of fly density on weevil distribution on a per head basis were determined at Rabbit Ridge and Heinz Cabin. Weevil attack was significantly negatively correlated with the number of fly galls present in the seed head ($n=5$, $r=-0.992$, $p=0.001$, Fig 1.4A; $n=5$, $r=-0.984$, $p=0.003$, Fig. 1.4B, Rabbit Ridge and Heinz Cabin respectively).

The competitive effect of the weevil on the fly was measured at Heinz Cabin by calculating the proportion of fly galls destroyed by the weevil in uncaged plants at two levels, the proportion of seed heads containing destroyed galls and the proportion of total galls destroyed. At the seed head scale, the proportion of seed heads attacked by both species which contained weevil-destroyed galls, are plotted in Figure 1.5A. At the scale of the gall, the proportion of destroyed galls in seed heads attacked by both species, as well as the weevil-destroyed proportion of total galls, are plotted in Figure 1.5B.

Additive Attack

The proportion of seed heads attacked by both species in combined treatments was significantly higher than the proportion of seed heads attacked by either species when released on their own at Wapiti Lake, Rabbit Ridge, and Heinz Cabin ($x^2=4.800$, d.f.=1, $p=0.028$, Fig. 1.6A; $x^2=5.138$, d.f.=1, $p=0.023$, Fig. 1.6B; $x^2=12.059$, d.f.=2, $p=0.002$, Fig. 1.6C, respectively). The proportion of seed heads attacked by the weevil, when it was released on its own, was significantly higher than the proportion of heads attacked by the fly when it was released on its own at Wapiti Lake and in the low fly release rate at Rabbit Ridge ($x^2=5.138$, d.f.=1, $p=0.023$, Fig. 1.6A; $x^2=9.267$, d.f.=1, $p=0.002$, Fig. 1.6B, respectively), but no significant difference was detected when compared to the high fly release rate at Rabbit Ridge or at Heinz Cabin ($x^2=0.199$, d.f.=1, $p=0.905$, Fig. 1.6b; $x^2=0.025$, d.f.=1, $p=0.874$, Fig. 1.6c, respectively), suggesting that the higher weevil attack rates at Wapiti Lake and the low fly release rate at Rabbit Ridge may be an artifact of the fly release rates selected for the experiment.

Because the combined treatments at Wapiti Lake and Rabbit Ridge had double the number of insects released into them compared to the single-species treatments, the proportion of heads attacked per insect released (attack efficiency) was calculated for each treatment. Attack efficiency was highest in the weevil-only treatment, followed by the fly-only treatment, and lowest in the combined treatments at both sites (Fig. 1.7A, B).

Seed Reduction

At Wapiti Lake, the proportion of seed destroyed in the weevil-only treatment was significantly higher than in the combined treatments ($x^2=5.138$, d.f.=1, $p=0.023$, Fig. 1.8A). The proportion of seed destroyed in the combined treatment was significantly

higher than occurred in the fly-only treatment ($\chi^2=33.683$, d.f.=1, $p<0.001$, Fig. 1.8A). Because the combined treatment had double the number of insects released into it relative to each of the single-species treatments, the proportion of seed destroyed per insect released (control efficiency) was calculated for each treatment. Control efficiency was highest in the weevil-only treatment, followed by the combined treatment, and lowest in the fly-only treatment (Fig. 1.8B).

Differences between expected and observed proportions of seed destroyed were significant for all possible within-head insect combinations in the combined treatment. The proportion of seed destroyed by the weevil was significantly lower than expected ($\chi^2=29.88$, d.f.=2, $p<0.005$, Fig. 1.9). Significant differences between expected and observed values were also detected for remaining seed in un-attacked heads as well as for heads that were attacked by the fly only ($\chi^2=12.9$, v=d.f., $p<0.005$, Fig. 1.9). In both cases, the observed values were higher than what was expected.

Seed reduction was also measured at Heinz Cabin. There was a significant decrease in remaining seed as the weevil to fly ratio increased ($n=66$, $r=-0.643$, $p<0.001$, Fig. 1.10).

CPR Experiment

There was no significant difference between the mean number of seed heads attacked by the fly at sites where the fly was released compared to sites where exclusion of the fly was attempted, suggesting that the release rates used for this experiment were too low to have an effect on existing populations ($t=1.330$, d.f.=237, $p=0.185$, Fig. 1.11). As a result, no further analysis was conducted for this experiment, even though the mean

number of weevils per seed head were significantly higher at the release sites compared to the non-release sites ($t=-3.648$, $d.f.=237$, $p<0.001$, Fig. 1.11).

Discussion

Interspecific competition is an important process operating on fly and weevil populations on spotted knapweed. The fly interferes with the weevil, causing significant population reductions compared to if the weevil was attacking on its own (Fig. 1.1), and the rate at which the two species co-occur within a spotted knapweed capitulum is significantly lower than expected (Fig. 1.3). The reduction in weevil attack rates was manifested in seed heads that would typically have contained both species (Fig. 1.3). These results on spotted knapweed are consistent with what has been found for these two species on diffuse knapweed (Seastedt et al. 2003). Zwolfer (1979) also reported a similar, negative interaction between *Larinus* weevils and *Urophora* flies on *Carduus nutans*. The fly in this case was also competitively superior, with the weevil being displaced from about 50% of flower heads.

Fly attack density per seed head was also an important aspect of the interaction. As fly density increased per cage at Heinz Cabin, the number of weevils successfully attacking within the cage decreased significantly (Fig. 1.2). Fly density significantly reduced weevil success at the seed head scale as well (Fig. 1.4). The effects of density were not consistent, however, over all experiments. No difference in weevil attack rates between low and high fly-release rates at the Rabbit Ridge cage experiment was detected; weevil attack rates were significantly reduced in both treatments (Fig. 1.1B). This may

be a function of the release densities selected, however, with even the low fly density being beyond the competitive threshold.

Although it is common for competition to vary in time and place (Connell 1983), the overall patterns in the present study were consistent in both years, across all experiments, and at all study sites. It was also detected in the presence of other, potentially overriding, factors, such as environmental heterogeneity, exposure to natural enemies, and intraspecific competition; factors that are commonly cited as suppressing insect herbivore populations below ‘competitive thresholds’ (Schoener 1983). There was variation, however, in the intensity of the competition, which was most likely a function of release densities and variable plant phenology.

Groppe (1990) predicted that the weevil would not have deleterious effects on other seed head agents and Harris (1989) argued that the hardened gall would protect the fly from other seed head feeders. While the weevil did destroy a portion of fly galls (Fig. 1.5), there was no significant effect on fly populations (Fig. 1.6C), suggesting that the overall interaction is strongly asymmetrical in favor of the fly. This asymmetrical interaction was not unexpected, however, as results from studies on interspecific competition seem to suggest that asymmetry is commonplace among pairs of competing species (Lawton and Hassell 1981, Connell 1983, Strong et al. 1984b, Denno et al. 1995).

Although displacement is a well-documented consequence of interspecific competition (see Reiz and Trumble 2002), total exclusion of the inferior competitor from the habitat is not a necessary requirement. Significant competitive effects may manifest themselves as reduced fitness or abundance (Abrams 1990). Lang et al. (2000) reported that the fly and the weevil were capable of co-existing. From the present study, the two

species do coexist; they are found together at a site and in a seed head, but their densities are not what would be expected if their distribution were random. The consequence of its interaction with the fly is that the weevil is being excluded from a portion of spotted knapweed seed heads. Interspecific competition may affect the abundance of these populations, but it does not seem to affect the species composition of the community.

As insect herbivore abundance increases during population outbreaks, the role of interspecific competition in population structure becomes more central (Menge and Sutherland 1987, Tomlin and Sears 1992, Begon et al. 1996). In its native range, the weevil attacks an average of 21.3% of spotted knapweed seed heads (Groppe 1990). In the present study, attack rates of spotted knapweed seed heads by the weevil were 72% when the fly was absent, and 41.5% on heads exposed to both species. The higher weevil attack rate, even with the competitive interaction with the fly, suggests a possible release of this species from indigenous regulating factors.

Seastedt et al. (2003) cites the weevil as the major contributor to diffuse knapweed declines and Lang et al. (2000) reports that the weevil, at high densities, was capable of causing significant plant destruction. Pearson et al. (2000) have noted that the fly used in this study has been found to achieve very high densities, and yet has not resulted in a reduction in spotted knapweed density (Pearson et al. 2000). In the context of seed reduction, the weevil was far more efficient at all of the research sites, where it contributed more to seed destruction than did the fly, and as a result, would be the more desirable of the two agents. Seed reduction was greatest when the weevil was attacking on its own, higher even than when both species were attacking the host simultaneously (Fig. 1.8, 1.9, and 1.10). The problem from an applied perspective is that the superior

biocontrol agent is the inferior competitor. Harris (1991) found the gall forming *Urophora* fly interfering with the weevil *Rhynocillus conicus* on *Carduus nutans*, even though the weevil was a better control agent (Zwolfer 1973).

Ehler and Hall (1982) and Myers (1985) hypothesize that introducing several agents could lead to reduced control because of competition. McFadyen (1998) argues that the idea has been disproved. The results described in these experiments support the hypothesis that competition can occur between biocontrol agents, and that competition can lead to reduced control of the target pest, and supports McEvoy and Coombs (1999), who have argued that the number of biocontrol species released against a particular host should be minimized, so as to reduce interference effects among biocontrol species.

The cumulative stress hypothesis holds that consumption of the weed will increase with the number of agents released, and assumes that attack from multiple agents is additive. There are several studies documenting such a response (Zwolfer 1985, Harris 1989, McEvoy et al. 1993, Fowler and Griffin 1995, Hallett et al. 1995). Harris (1989) predicted that attack rates on spotted knapweed achieved by the fly would be supplemented by introducing the weevil, in spite of reports that insects which attack early in the season and have an aggregated distribution, like that of the fly, tend to achieve higher levels of resource exploitation. In such circumstances, increasing the number of insect species released as agents would increase exploitation levels only marginally (Zwolfer 1985, 1988). My data do demonstrate some measure of additive attack, with a greater proportion of seed heads being attacked with both species present, compared to attack rates of any single species on its own (Fig. 1.6), and improved rates of seed reduction compared to pre-release levels (see also Lang et al. 2000). This study also

illustrates, however, that levels of seed reduction are lower when both species are present at a site compared to what would be achieved with only the weevil.

Although seed reduction caused by insect herbivores is quantifiable, the debate as to whether or not seed predators can regulate host plant population dynamics remains unresolved (Crawley 1989b, McEvoy 2002 and references therein). Even for plant species that reproduce primarily by seed, plant populations are not necessarily seed limited. The consequence from a biological control perspective is that even though an insect species, or combination of species, may significantly reduce seed output, there still may be no effect on plant population dynamics (Crawley 1989b, Muller-Scharer and Schroeder 1993). Rosette survival is reported as being the critical stage in spotted knapweed life history (Muller 1989b). As De Clerck-Floate and Bouchier (2000) have noted, the effect of a biocontrol agent on plant population dynamics must play a role in the decision to release it (De Clerck-Floate and Bouchier 2000). Even for host specific biocontrol agents, consequences of introductions that provide little or no control of the pest population may include dramatic non-target community effects (Pearson et al. 2000) and the stimulation of compensatory mechanisms that make the target weed even more competitive (Calloway et al. 1999).

The spotted knapweed system illustrates the importance of more thorough pre-release studies and the integration of ecological knowledge with biocontrol practices. The fly in this study is a biocontrol agent that has achieved a high biomass, has not had any tangible impact on knapweed population dynamics, can displace a superior agent, and has had important non-target effects on native communities (Story et al. 1995, Pearson et al. 2000).

Chapter 1 Figures

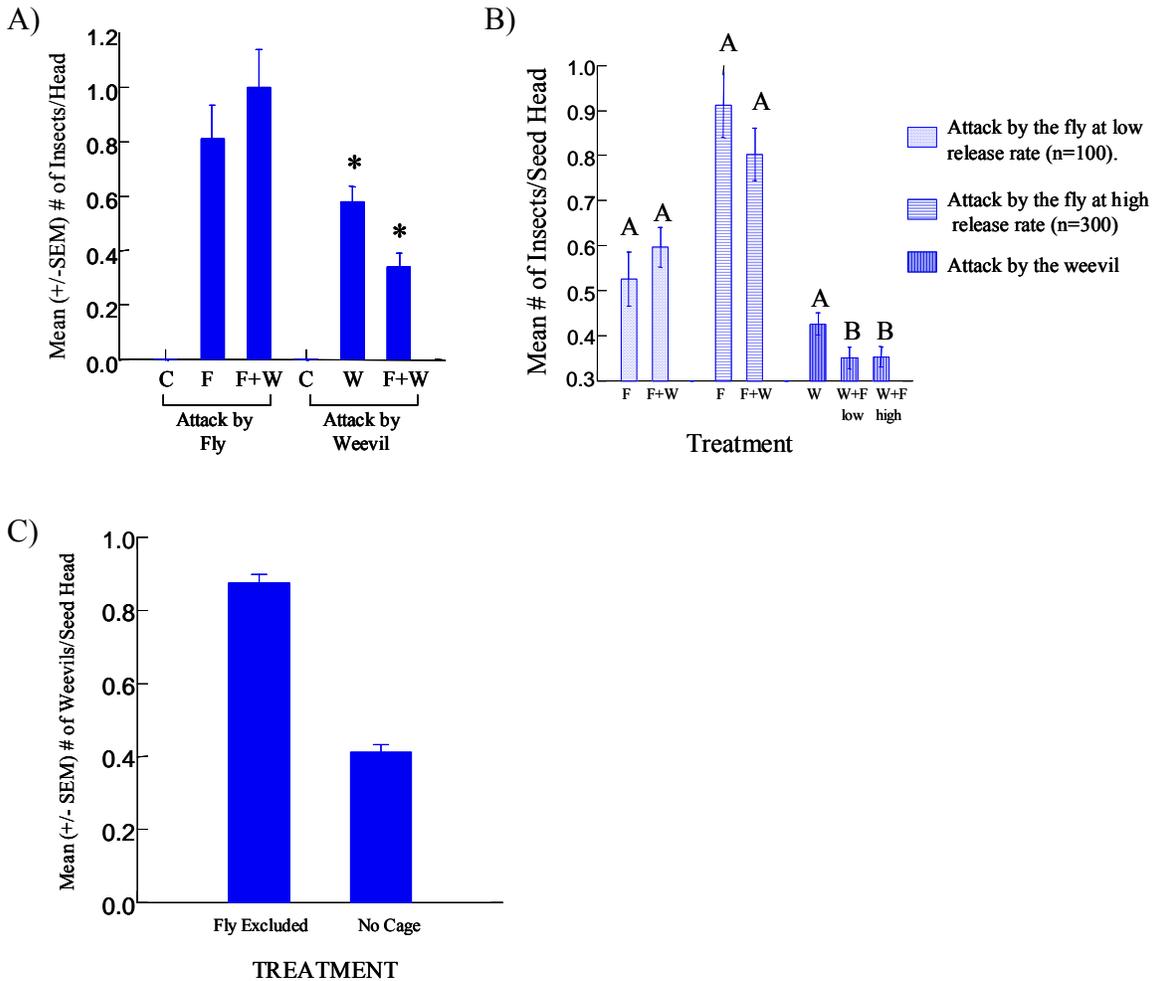


Figure 1.1. Mean (+/- SEM) number of flies and weevils per spotted knapweed seed head in each experimental treatment at **A)** Wapiti Lake 2001. Differences in means were analyzed with two nested ANOVA's with treatment as the fixed effect and enclosures nested within treatment. C=control, F= Fly only treatment, W= Weevil only treatment, and F+W= treatment with both species released together. * Indicates a significant difference detected by Tukey's post-hoc test ($F=58.912$, $d.f.=3,4,382$, $p<0.001$). Although means (+/- SEM) is presented in the graph, the analysis was done on Least Square Means. **B)** Rabbit Ridge 2002. Differences in means were analyzed by two 1-way ANOVA's with treatment as the factor. F= fly only, W= weevil only, and F+W = combined treatment. Different letters indicate significant differences detected by Tukey's post-hoc test ($F=3.117$, $d.f.=2,289$, $p=0.046$). **C)** Heinz Cabin 2002. Differences in weevil attack per spotted knapweed seed head on plants that have been exposed to attacking populations of both species for the entire season (No Cage) and plants on which the fly has been excluded. The result of a T-test indicate a significantly higher weevil attack rate when the fly had been excluded ($t= 9.724$, $d.f.=488$, $p<0.001$).

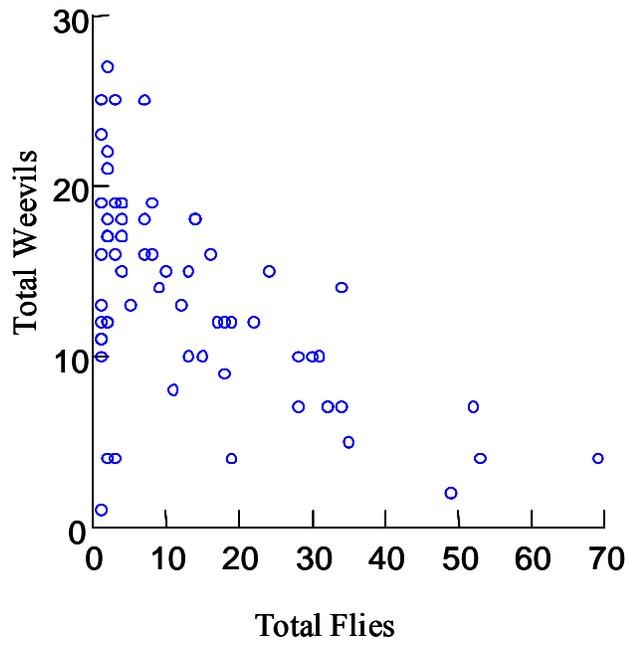


Figure 1.2. The relationship between the total number of flies and the total number of weevils attacking seed heads within each experimental cage at Heinz Cabin 2002. Totals were log-transformed for the correlation analysis ($n=66$, $r=-0.597$, $p=0.003$).

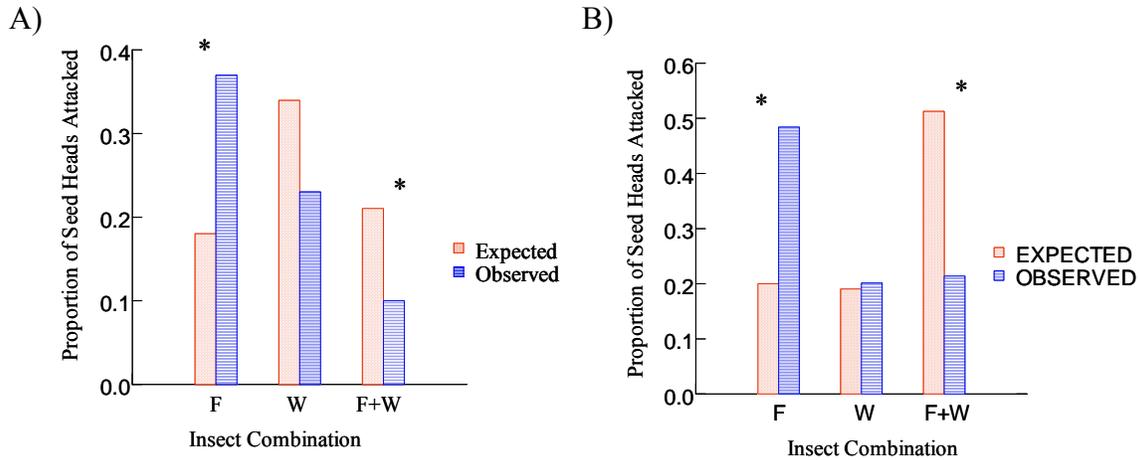


Figure 1.3. Comparison between expected and observed fly and weevil distribution on spotted knapweed seed heads in the combined treatment at **A)** Wapiti Lake 2001 and **B)** Heinz Cabin. Expected values for attack pattern in combined treatments were obtained from observations in the single-species treatments. Observed values were obtained by dissecting seed heads from the combined treatment only. F+W= heads attacked by both species, F = heads attacked by the fly only, and L = heads attacked by the weevil only. * Indicates a significant difference between expected and observed values (**A**: $\chi^2=28.51$, d.f.=3, $p<0.05$ for F; $\chi^2=8.45$, d.f.=2, $p<0.05$ for F+W, and $\chi^2=2.69$, d.f.=1, $p>0.05$ for W; **B**: $\chi^2=55.62$, d.f.=3, $p<0.05$ for F, $\chi^2=16.42$, d.f.=2, $p<0.05$ for F+W; and $\chi^2=0.55$, d.f.=1, $p>0.05$ for W).

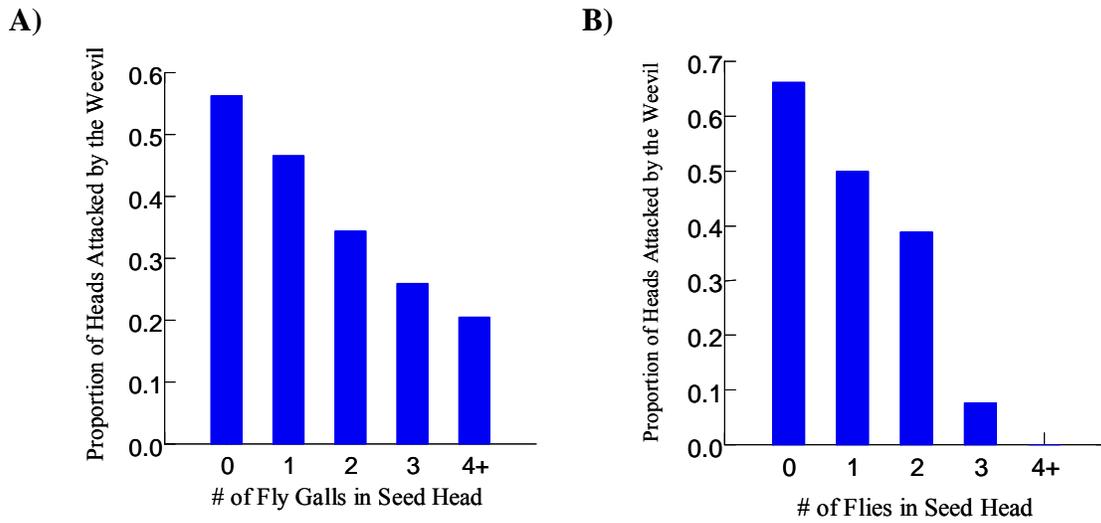
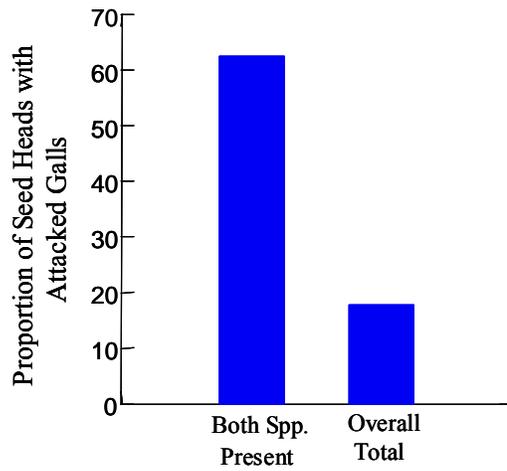


Figure 1.4. The relationship between the proportion of spotted knapweed seed heads attacked by the weevil within and the fly frequency distribution in **A)** the high release-rate combined treatment at Rabbit Ridge 2002 ($n=5$, $r=-0.992$, $p=0.001$), and **B)** un-caged plants at Heinz Cabin 2002 ($n=5$, $r=-0.984$, $p=0.003$).

A)



B)

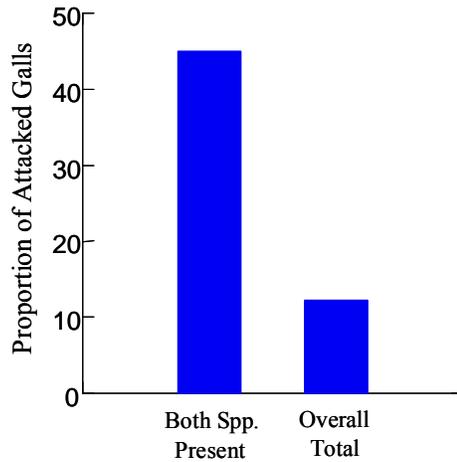


Figure 1.5. The effect of the weevil on the fly at Heinz Cabin 2002. **A)** The proportion of seed heads in which the weevil had destroyed fly galls in seed heads attacked by both species (Both Spp. Present), and as a proportion of the total number of seed heads dissected (Overall Total). **B)** The proportion of fly galls destroyed by the weevil in seed heads attacked by both species (Both Spp. Present) and as a proportion of the total number of galls observed (Overall Total).

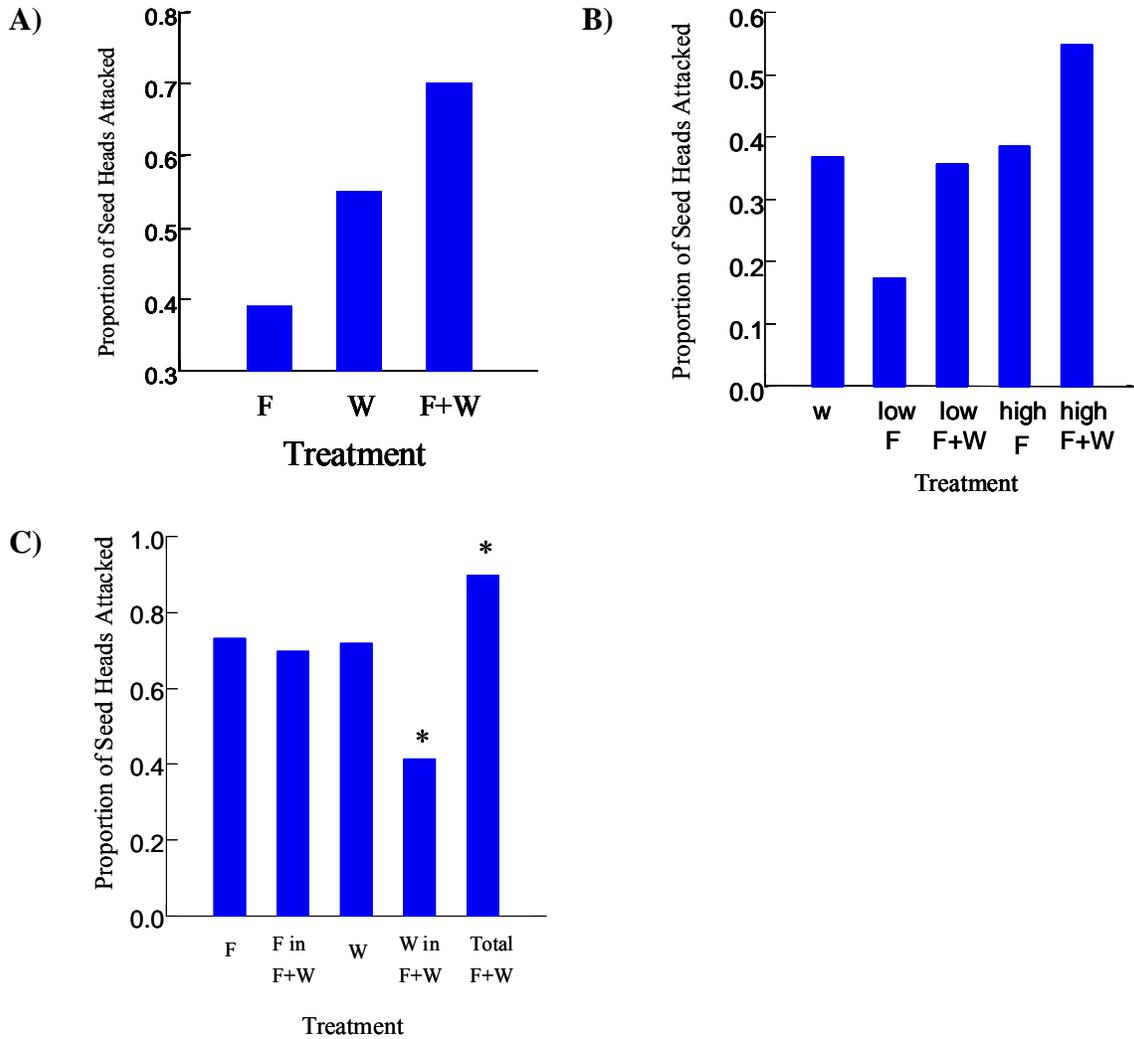


Figure 1.6. Proportion of spotted knapweed seed heads attacked in each of the experimental treatments. Data were analyzed with contingency tables and each contrast was interpreted using Bonferroni's correction. F=Fly, W=Weevil. **A)** At Wapiti Lake, a significant difference in attack rates between fly-only and weevil-only treatments ($\chi^2=5.138$, d.f.=1, $p=0.023$) was detected. Attack rates in weevil-only and combined treatments were not statistically different ($\chi^2=4.800$, d.f.=1, $p=0.028$). **B)** At Rabbit Ridge, the attack rate in the high combined treatment was significantly higher than any of the other treatments ($\chi^2=5.138$, d.f.=1, $p=0.023$), the attack rate in the low-Fly treatment was significantly lower than any other treatment, ($\chi^2=9.267$, d.f.=1, $p=0.002$), and the attack rates in the remaining three treatments were not significantly different ($\chi^2=0.199$, d.f.=1, $p=0.905$). W = weevil only treatments, low F = fly only, low release rate (n=100), high F = fly only, high release rate (n=300), low F+W = fly (low release rate) + weevil, high F+W = Fly (high release rate) + Weevil. **C)** At Heinz Cabin. * Indicates a statistically significant difference after the Bonferroni correction ($p<0.0025$).

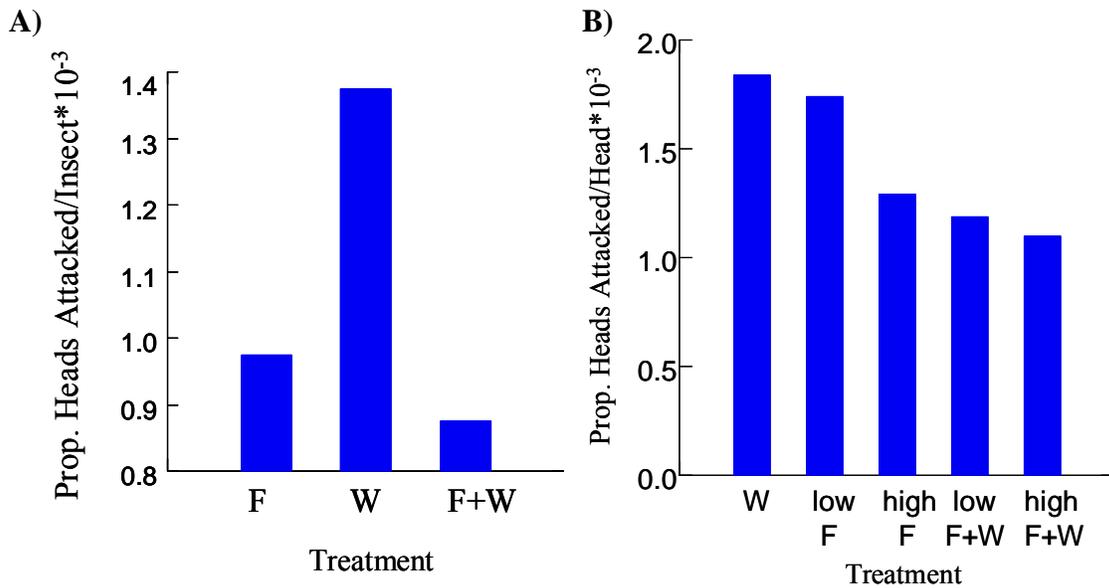


Figure 1.7. The proportion of spotted knapweed seed heads attacked per insect released for each experimental treatment. Data were not analyzed statistically, but were included to demonstrate that the higher attack rates in the combined treatment (see Figure 6) were an artefact of the release rate, and that attack efficiency decreased when both species were released together. **A)** From Wapiti Lake. F = fly only, W = weevil only, F+W = both species released. **B)** From Rabbit Ridge. W = weevil only treatments, low F = fly only, low release rate (n=100), high F = fly only, high release rate (n=300), low F+W = fly (low release rate) + weevil, high F+W = Fly (high release rate) + Weevil.

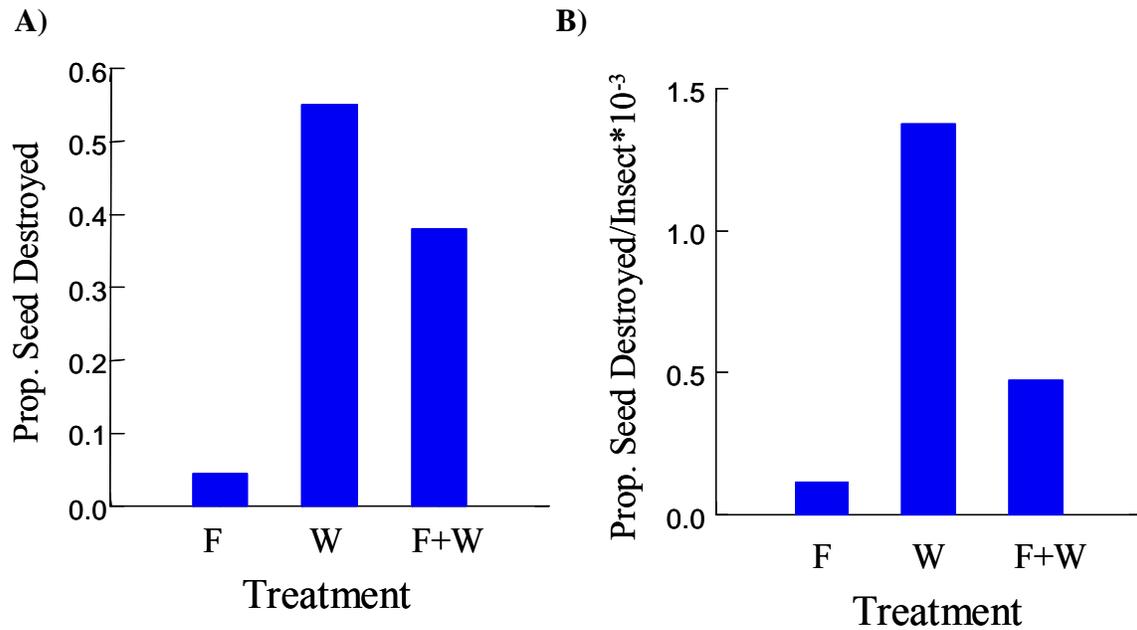


Figure 1.8. **A)** Proportion of spotted knapweed seed destroyed at Wapiti Lake 2001 by either or both insect species for each of the experimental treatments. Data were analyzed with contingency tables and interpreted using Bonferroni's correction. A significant difference in attack rates between fly-only and the combined treatments ($\chi^2=33.683$, d.f.=1, $p<0.001$), as well as between weevil-only and combined treatments ($\chi^2=5.138$, d.f.=1, $p=0.023$) was detected. **B)** The proportion of spotted knapweed seed destroyed corrected for the release density in each of the treatments. F = fly only (n=100 heads), W = weevil only (n=100 heads), F+W = both species released (n=100 heads).

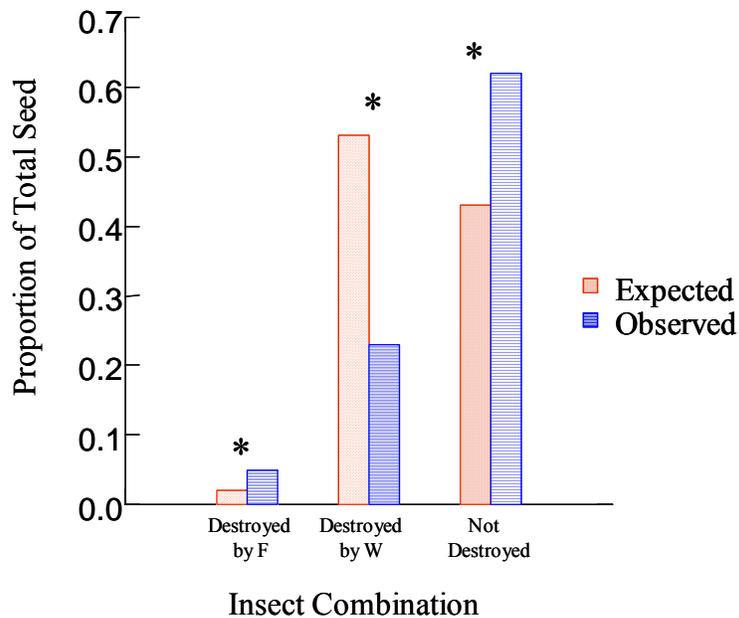


Figure 1.9. Comparison between expected and observed seed reduction within spotted knapweed seed heads in the combined treatment at Wapiti Lake 2001. Values along the y-axis refer to the expected and observed proportions of total seed within the combined treatment, either destroyed or not destroyed. Expected values were obtained from data collected in the single-species treatments and observed values were obtained from the combined treatment. Destroyed by F = proportion of total seed destroyed in heads attacked by the fly only, Destroyed by W = proportion of total seed destroyed in heads attacked by the weevil only, and Not Destroyed = proportion of total seed not destroyed. * Indicates a significant difference between the expected and observed values ($p < 0.05$).

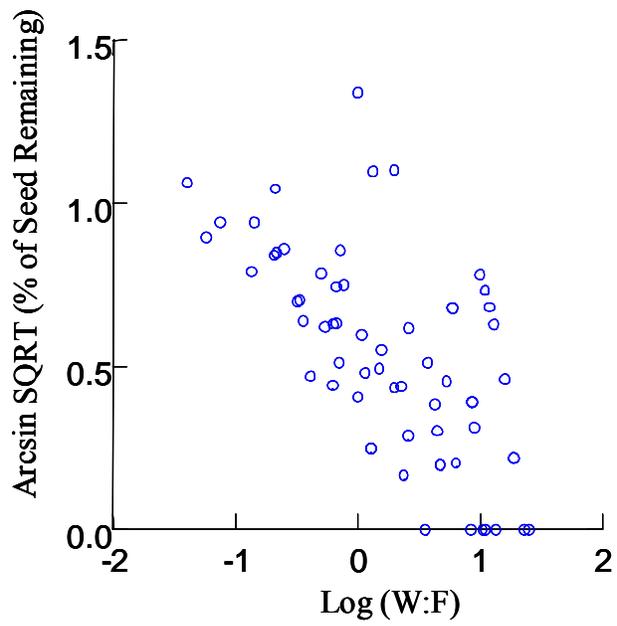


Figure 1.10. The relationship between the weevil to fly (W: F) ratio and the proportion of seed remaining within the experimental cages at Heinz Cabin 2002 (n=66, $r=-0.643$, $p<0.001$).

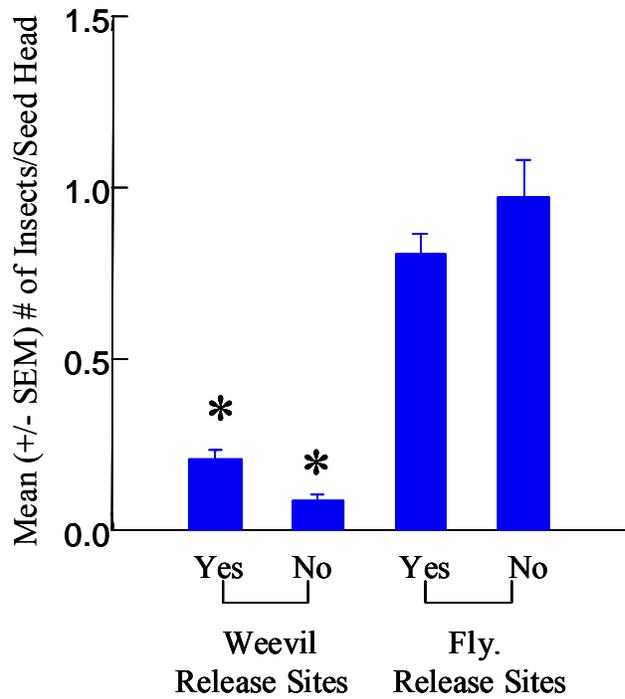


Figure 1.11. The effect of insect release on mean (+/- SEM) numbers of weevils and flies per spotted knapweed seed head at CPR release sites in 2002. * Indicates a significant difference in mean number of attacking weevils between release and non-release sites ($t=-3.648$, $d.f.=237$, $p<0.001$).

Chapter 2: Plant-mediated mechanisms of interspecific competition between two insect herbivore biocontrol agents.

Abstract

The role of spotted knapweed phenology on insect herbivore attack density was assessed in a series of manipulative and observational studies. The implications of plant phenology-induced variation in insect density are evaluated with respect to interspecific competition between the fly and the weevil. Hypotheses of four plant-mediated mechanisms of interspecific competition are also tested. These hypotheses included resource preemption, space preemption, differential resource acquisition, and overgrowth. Results suggest that plant phenology has an important role for attack densities achieved by the insect herbivores, and subsequently, for interspecific competition as well. Results are best described by the resource preemption mechanism, and there was no support for either differential resource acquisition or space preemption, and although the overgrowth mechanism was observed, its effects were not significant.

Introduction

In addition to understanding horizontal interactions between biocontrol agents, vertical interactions are also of interest to ecologists and biocontrol practitioners, particularly if the objectives of biocontrol programs are to be realized. Classical weed biocontrol removes top-down regulation on insect herbivores, which may result in outbreak densities. In such circumstances, insect herbivores could have the potential to significantly reduce their host plant populations (Moran and Hoffmann 1989). However, evidence suggests that bottom-up regulation of insect herbivore populations can play a dominant role, even in biocontrol systems (Hunter and Price 1992, Floate and De

Clerck-Floate 1993). For example, biological weed control with insects is characterized by high rates of insect establishment, and rapid increases in insect density, but with little or no control of the target weed where tested (Myers 1985, Crawly 1989). This has been attributed to the fact that many promising insects, once introduced into the new environment, fail to meet expectations (Ewel et al. 1999). In circumstances where the insect fails to regulate weed populations, bottom-up plant-insect interactions may operate with greater prevalence and with greater consequence to insect herbivores (Raven et al. 1999). Crawley (1989b) concluded that, ‘overall, plants have more impact on herbivores than herbivores have on plants’.

Variation in host-plant phenology has been documented as having a central role for insect abundance (Faeth et al. 1981, Pritchard and James 1984, Potter 1985, West 1985, Hunter 1990, 1992, Auerbach 1991, Hunter and Price 1992). This is especially true for specialist herbivores, which often attack the plant at a specific stage of development (Kearsley and Whitham 1989, Quiring 1992). Close synchrony between an insect and the resource dynamics of its host can lead to increased insect densities, whereas consequences of asynchronization between insect and plant include reduced insect density (Turgeon 1986).

Variation in host plant phenology can also be critical to insect population processes such as interspecific competition (Muller 1989, Hunter 1992). Its role can be particularly important when plant-insect synchronization results in outbreak densities (Seifert and Seifert 1976). Hunter (1990) reports how asynchronization events can reduce population density and limit competition, whereas close synchronization results in higher population densities and more intense competition. Variation in plant phenology

implies variation in the temporal availability of plant resources that are required by insect herbivores. One consequence of having a portion of resources unavailable to attacking insects, and a portion of resources that are, is that insect attack patterns may reflect more of an aggregated distribution, increasing both the frequency and intensity of interspecific competition on resources where aggregation occurs. Under such circumstances, interspecific competition may play a dominant ecological role, even though resources have the perception of being relatively abundant (Mattson 1986, Crawley and Pattrasudhi 1988, Hunter and Willmer 1989, Rieder et al. 2001).

The results from Chapter 1 indicate that the fly is interfering with the weevil. Inasmuch as competition is a density-dependent process, factors contributing to fly density become important considerations with respect to the frequency and intensity of the competitive consequences for the weevil, and are the focus of this chapter. Variation in host plant phenology may have important consequences for attack densities achieved by the fly, as has been demonstrated for other gall forming insects. If host plant phenology varies such that fly attack densities are limited, competition between the fly and the weevil would be infrequent and may be unimportant. If, however, plant phenology is such that the fly is capable of achieving high attack densities, competition may play an important role, significantly reducing weevil abundance.

Plant-Mediated Mechanisms of Interspecific Competition

Schoener (1983) and Reiz and Trumble (2002) describe several mechanisms of interspecific competition, including four that are relevant to the spotted knapweed system. These four mechanisms are resource pre-emption (Reiz and Trumble 2002), space pre-emption (Schoener 1983), differential resource acquisition (Reiz and Trumble

2002), and overgrowth (Schoener 1983). Because temporal variation in both the suitability of seed heads and the availability of seeds can drive insect attack and subsequent interactions, each of these mechanisms are considered to be plant-mediated for the knapweed system.

Resource Preemption

Resource pre-emption occurs when one species utilizes a critical resource before it becomes available to a competitor (Reiz and Trumble 2002). The role of this mechanism between the fly and the weevil is potentially great, with the fly attacking the seed head at an earlier stage of seed head development than the weevil. The gall initiated by the fly on knapweed acts as a metabolic sink, utilizing plant nutrients that would otherwise be invested into seed production (Harris 1980). These developing seeds are a critical resource for newly hatched weevil larvae that occur in the seed head later in the season, after gall initiation (Groppe 1990). Harris (1989) reported that the gall was such a powerful sink that it was capable of preventing the plant from flowering at all. The consequence of such a phenological disruption would be severe for the weevil, given its ovipositional requirement of a flowering seed head. If resource pre-emption is occurring in this system, the weevil would either experience a shortage of food immediately upon larval emergence and mortality rates at this particular stage of development would be considerably high, or it would not be able to attack the plant at all on account of a gall-induced disruption to flowering phenology.

Space Preemption

Space pre-emption occurs when a member of one species occupies a unit of space prevents an individual of another species from occupying that space (Schoener 1983).

On knapweed, the fly attacks the seed head at an earlier phenological stage of development than does the weevil. The consequence for an ovipositing weevil is that a gall may already be established in a seed head, occupying space that may be required for the development of the weevil larva. Harris (1991) predicted that once the fly gall hardens, the seed head would be avoided by other species. If this mechanism of competition were operating between these two species, the weevil would be avoiding heads already attacked by the fly, and weevil oviposition would only be observed on seed heads not attacked by the fly.

Differential Resource Acquisition

Differential resource acquisition occurs when individuals from one species are able to obtain a sufficient portion of required resources while individuals of another species are not (Reiz and Trumble 2002). The implication is that both species co-occur and compete for a resource that is in limited supply. On spotted knapweed, both the fly and the weevil are developing as larvae at the same time, presumably in the same seed heads, and both utilizing a limited supply nutrients invested in seed production. Were this mechanism operating, the superior competitor would garner sufficient resources, while the inferior competitor would suffer either increased mortality or decreased growth rate.

Overgrowth

Overgrowth occurs when one species grows over or upon a second species and causes harm to that individual in the process (Schoener 1983). Although this mechanism has yet to be demonstrated in animal populations (Schoener 1983), it may be occurring between the fly and the weevil on knapweed because of the rapid larval development of

the weevil and its tendency to utilize the entire contents of seed head in the construction of its pupal chamber (Groppe 1990). The weevil has not been reported to predate upon other members of the seed-feeding guild, but may inadvertently destroy the gall and fly during the formation of its pupal cell. If this mechanism is operating with a significant effect on fly populations, fly density should significantly decrease as the weevil enters the pupal stage of development.

The objectives of the experiments described in this chapter are to: 1) evaluate the role of individual host-plant phenology on the attack rates of the fly, and then to investigate the implications of fly density on the nature of the competitive interactions between the fly and the weevil, and 2) to determine which, if any, of the aforementioned plant-mediated mechanisms of competition are operating in the interspecific interactions between these two species of weed biological control agents on spotted knapweed.

Materials and Methods

Plant and Insect Phenology

Spotted knapweed phenology and adult insect recruitment for the fly and the weevil were monitored from June 5th to August 28th, 2002 at four sites in the west Kootenay region of southeastern British Columbia. The four sites were Heinz Cabin, Seven-Mile Reservoir, Brilliant, and Sanca Creek. All four sites were dominated by a well-established spotted knapweed monoculture and each had well-established populations of both the fly and the weevil. At each site, a sampling grid was implemented that consisted of five transects, equidistant apart, each running the length of the patch. The length of each transect, and the distance between transects, varied

between sites according to the dimensions of the patch. Along each transect, six sampling points, equidistant apart, running the length of each transect, were established, for a total of 30 sampling points per site, each used to monitor insect recruitment by collecting and counting adults with a sweep net. Plant development was assessed using six randomly selected plants located between transects, for a total of 30 plants.

Insect recruitment was sampled on a weekly basis at each sampling point. Both species are typically distributed on the upper portion of the host plant, and both are easily captured with a sweep net. At each sampling point, the sampler would make two passes through the spotted knapweed vegetative growth with the sweep net, each pass having a sweeping-radius of approximately 1m. Captured adults of both species were identified, counted, and released back into the patch.

Spotted knapweed phenology was measured each week by classifying seed head development on a per plant basis using the 30 plants at each of the four sites. The plant flowers indeterminately, and monitoring of seed head development commenced once the plants had bolted and bud development had initiated, June 18th, and continued until August 28th, 2002. During the weekly sample, each seed head on each plant was categorized into one of six distinct development stages (Turgeon 1986, Crawley and Akhteruzzaman 1988, Quiring 1992, Floate 1993, Connett et al. 2001). Developing seed heads were categorized according to the following schedule: 1. Bud Initiation, 2. Bud Elongation, 3. Pre-flowering, 4. Flowering, 5. Wilted Florets, and 6. Seed Set. The phenology data permitted the proportion of seed heads at any given stage during any week of the study to be determined. This value was used as a measure of plant phenology at the both the plant and patch levels.

Plant Phenology and Insect Attack Densities

The study site was located at Heinz Cabin in the Pend d'Oreille valley in southern British Columbia. The site is described in Chapter 1.

Ten transect lines, each spaced five meters apart, were established along the length of the patch, which was approximately 75 m. Along each transect, eight cages were randomly placed over clusters of bolting spotted knapweed stalks. The number of stalks per cage ranged from one to 12. The cages were fashioned of finely meshed aluminium screen (1.5 m x 1 m strips) stapled to two 36-inch wooden stakes and sealed at the top. All old stalks were removed from within each cage to prevent over-wintering flies from emerging into the cage.

The relationship between fly oviposition and host plant development was determined by exposing a cohort of ten cages to attack from the established fly population for a period of one week, and then replacing the cages at the end of the week to prevent any additional attack. Weekly cohorts covered the entire flight period of the fly, from June 10 to July 31, 2002. Ten control cages were also placed on clusters of knapweed stalks at the beginning of the experiment and were not removed.

All of the plants were collected from within the cages on August 28, 2002, and 20 seed heads per cage were dissected. The 20 uppermost seed heads from each cage were selected for dissection to ensure that heads of similar phenological age were being used for between-cage comparisons. The number of flies attacking spotted knapweed seed heads in each of the weekly cohorts were counted. Weekly oviposition rates were then correlated with the proportion of seed heads at the stage required by the fly for

oviposition (stage 2), as measured by the phenological categorization method for seed head classification described above.

Weevil attack phenology was assessed by randomly collecting 15 spotted knapweed plants from within the same patch on a weekly basis from June 10 to August 28, 2002. All of the seed heads occurring on the sampled plants were dissected in the laboratory and all of the weevil eggs within each seed head were identified and counted. Synchronization between the weevil and its host plant was measured as the correlation between weekly oviposition rates and the proportion of seed heads at the development stage required for weevil oviposition (stage 4) at each week.

On the week of August 26, 2002, when the adult phase of populations of both insect species was complete, 15 of the 30 plants selected for phenological monitoring were harvested from each of the four monitoring sites and brought into the laboratory where all of the seed heads on each of the plants were dissected, and eggs, larvae, and pupae of each species were identified and counted.

For each of the four sites, the proportion of seed heads at each of the six development categories was tallied for each week of the monitoring period and used as a measure of plant phenology for any point in time throughout the field season. To determine the role plant phenology had on observed fly attack rates, the mean number of flies per seed head was obtained for each plant and then compared to the proportion of seed heads on those same plants that were at stage 2 of development during the peak two week period of fly oviposition. Similarly, the role of plant phenology on weevil attack rates was determined by comparing the proportion of seed heads on each plant that were attacked by the weevil to the proportion of seed heads on each plant that were at stage 4

during the peak two week period of weevil oviposition. This two-week period of peak oviposition occurred one week after the population of each species had peaked at Heinz Cabin, and this observation was used to estimate the two-week period of peak oviposition at the other three sites. The relationship between attack rates of each species and plant phenology at each of the four monitoring sites was analyzed using a correlation between phenology and arcsin square root transformed proportions of attacked seed heads.

Plant Phenology and Interspecific Competition

The role of plant phenology on competition was tested by collecting a weekly sample of 15 spotted knapweed plants from the Heinz Cabin site described above throughout the course of the season, from the week of July 2, 2002 to the week of August 28, 2002. Temporal variation in co-occurrence rates from the seed head dissections were used as a measure of the effect of plant phenology on insect distributions. The proportion of seed heads in which both species were co-occurring was compared to expected distributions that assumed random association and analyzed using a goodness of fit test (Stiling and Strong 1983, 1984).

Resource Pre-emption

Resource pre-emption was assessed by evaluating insect-induced disruptions to flowering phenology and by analyzing weevil mortality patterns following oviposition. The effect of attack by the fly on seed head development of its host plant was assessed in two separate experiments. First, by measuring seed head development over a season on plants kept within enclosures with fly and no-fly treatments. Second, by examining the time interval between peak-fly oviposition and the flowering phase of the host plant at two sites with relatively high fly densities.

The study site for the first experiment was located at Rabbit Ridge, near Elko, British Columbia, and is described in Chapter 1.

Four enclosure cages were placed into an existing patch of spotted knapweed. Each cage measured 5 ft w x 5 ft l by 7 ft h and was covered with a fine mesh screening. Old seed heads were cleared from within each cage so as to prevent over-wintering individuals from established fly populations from emerging into the experimental cages. Two replicates of four treatments were assigned to the cages, a control treatment in which no flies were released, and a treatment into which 300 adult flies were released. Adult flies were collected from a nearby population with a sweep net and introduced into the cages on July 4, 2002, which coincided with the peak flight period for that species.

The phenological stages of 30 plants in each of the two treatments were classified on a weekly basis throughout the summer according to the phenological categorization method described above. Mean phenological stages were determined per plant per week and comparisons between treatments were made using a two-way ANOVA with treatment, date, and treatment*date as factors.

The second experiment designed to evaluate the affect of fly attack on host plant phenology occurred at two of the four monitoring sites with relatively high, naturally occurring fly populations. At each site, the time from peak fly oviposition to peak flowering was determined on 15 of the 30 plants that were selected for phenological monitoring. This measure was then compared to the mean number of flies per seed head on each plant and the relationship was analyzed using correlations between the time from stage 2-4 for each plant and the mean number of flies per seed head.

Space Pre-emption

Weevil avoidance of fly-infested seed heads was assessed using the seed head dissection data from the weekly sample of 15 spotted knapweed plants from Heinz Cabin. The presence of weevil eggs in spotted knapweed seed heads was used as evidence of the weevil attacking a particular seed head. As seed heads were dissected, each weevil egg encountered was counted and scored as either being in a head containing one or more fly galls, or as being in a head on its own. The numbers of fly galls were also counted in each dissected seed head. The expected and observed egg distributions of the weevil were then compared to each other across the frequency distribution of the fly. The observed and expected weevil egg distributions with respect to the within-head fly frequency distribution were analyzed using a goodness of fit test. These were then compared to observed and expected adult emergence distributions at the end of the season with respect to the frequency distribution of the fly.

Differential Resource Acquisition

Weevil and fly attack dynamics were followed over the course of the season using the seed head dissection data from the weekly sample of 15 spotted knapweed plants from Heinz Cabin. The data were used to evaluate the differential resource acquisition mechanism in two ways, both focusing on mortality effects. First, if this mechanism were acting on these populations, a relatively high degree of co-occurrence would be observed early in the season, but as the superior competitor garners resources at the expense of the other species, a decline in the population of the inferior competitor over the course of the season would be expected. The change in the proportion of seed heads

containing flies and weevils was monitored over the season and analyzed using two-way contingency tables.

The second way in which the role of this mechanism in the competitive interaction between the weevil and the fly was evaluated was by comparing the distribution of emerged adult weevils with respect to the fly frequency distribution. If the mechanism were operating, weevil emergence in heads unoccupied by the fly would be higher than weevil emergence in heads containing fly galls. Correlations were used to analyze the relationship between adult weevil distributions and within-head fly frequency distributions.

Overgrowth

The overgrowth mechanism of competition was assessed using the seed head dissection data from the weekly sample of 15 spotted knapweed plants from Heinz Cabin. The larval stages of weevil development are rapid, taking approximately four weeks to complete (Groppe 1990). The fly remains in the larval stage within the gall for the entire period of weevil larval development. As the weevil prepares for pupation, it constructs a pupal chamber out of the post-feeding remnants of the seed head. It is during the formation of the pupal cell by the weevil that the overgrowth mechanism may play a role, as developed fly galls are potentially utilized as aggregate for the cell. The number of destroyed fly galls was counted in seed heads in which the weevil was present over the course of the field season. The number of intact galls per seed head were also counted at weekly intervals from the week of peak weevil oviposition, August 11, 2002, to the end of the season, the week of August 25, 2002, and in an additional sample of seed heads taken once the weevil had completed its development and had emerged from the seed

heads, the week of October 13, 2002. The proportion of seed heads containing destroyed galls destroyed over time was analyzed using two-way contingency tables, and was compared to the development of the weevil from the egg to the pupal stages.

Multivariable Hypothesis Testing

Logistic regression was used to assess the relative magnitude of the two variables of interest, plant phenology and fly density, on the two-state probability of the weevil either attacking a spotted knapweed seed head or not attacking it.

Results

Plant and Insect Phenology

Spotted knapweed phenology and adult insect recruitment for the fly and the weevil at each of the four monitoring sites are plotted in Figure 2.1. The development patterns of stage 2- and stage 4-category seed heads, the preferred development stages for fly and weevil oviposition respectively, were similar at all four sites. Insect recruitment patterns were also similar at each site, with significant temporal overlap of adults of both species. Adult weevils emerged from over-wintering sites in the soil litter at about the same time as adult flies began to emerge from the previous years' seed heads, which coincided with the bolting phase of plant development. Both insect species are abundant before the host plant was at their preferred stage for oviposition. This pattern was most pronounced for the weevil, which oviposits into seed heads at the flowering phase of development (stage 4), several weeks after the bolting phase had commenced and its emergence.

Although there were similar temporal recruitment patterns at each of the monitoring sites, adult insect densities were variable across the four sites. Site 4 had the highest adult fly population, followed by Site 1 and Site 2, with Site 3 having the lowest (Figure 2.2A). The highest adult weevil population was at Site 2, followed by Site 3 and Site 1, with Site 4 having the lowest (Figure 2.2B). Site differences in density would be an important consideration when analyzing a density-dependent process like interspecific competition. For example, site 4 had the highest fly and lowest weevil densities, and the two highest fly density sites were the two lowest weevil density sites (Fig 2.2).

Plant Phenology and Insect Attack Densities

Fly oviposition was significantly correlated with the proportion of seed heads available at the required stage ($r=0.778$, $d.f.=1$, $p=0.014$, Fig. 2.3). The same pattern was observed for weevil oviposition and the proportion of seed heads at the flowering phase of host plant development ($r=0.865$, $d.f.=1$, $p=0.006$, Fig. 2.4). Oviposition was not significantly correlated with any other stage of spotted knapweed development for either of the two insect species.

Cumulative fly attack rates at the end of the season were significantly correlated with plant phenology at site 1: $n=15$, $r=0.723$, $p=0.002$, Fig. 2.5; site 2: $n=15$, $r=0.748$, $p=0.002$, Fig. 2.5; and site 3: $n=15$, $r=0.583$, $p=0.023$, Fig. 2.5. There was no significant relationship at site 4 ($n=15$, $r=0.412$, $p=0.127$, Fig. 2.5), where all plants had a relatively high proportion of seed heads at suitable stage for fly oviposition.

There was also a relationship between the proportion of seed heads at stage 4 at peak weevil oviposition and the proportion of seed heads attacked by the weevil at the end of the season (Fig. 2.6). Site 1 ($n=15$, $r=0.859$, $p<0.001$); site 3 ($n=15$, $r=0.671$,

$p=0.006$); and site 4 ($n=15$, $r=0.758$, $p=0.001$) were all significant, whereas there was no relationship at site 2 ($n=15$, $r=0.077$, $p=0.795$). Site 2 did have the highest weevil density of all sites (Fig 2.2B), which may have contributed to the observed pattern, providing there were a sufficient number of weevils present to extend peak oviposition beyond the assumed two-week window.

Plant Phenology and Interspecific Competition

Over the course of the first part of the season at Heinz Cabin, from the week of July 21, 2002 to the week of August 4, 2002, there were significantly more heads attacked by both species than was expected, assuming distribution of both species was random (Fig. 2.7). This pattern disappeared by the week of August 11, 2002 and was reversed by the final sampling date, which took place the week of October 13, 2002. The lower co-occurrence than expected from the final sample was not significant at $\alpha=0.05$, but was at $\alpha=0.1$, and may illustrate biologically significant reduction in co-occurrence. Both species had an aggregated distribution to phenologically suitable plant parts at the beginning of the season, which appears to have intensified the competitive interaction such that by the end of the season, rates of co-occurrence were lower than expected.

Resource Pre-emption

Flies did not appear to alter resource suitability for the weevil. There was no significant effect of fly attack on plant phenology detected at either the Rabbit Ridge experiment ($F_{\text{trt}*\text{date}}=0.624$, d.f.=7,464, $p=0.736$, Fig. 2.8) or from the data collected at the two monitoring sites with the highest fly densities (Site 1: $n=15$, $r=0.077$, $p=0.781$, Fig. 2.9; Site 4: $n=15$, $r=0.241$, $p=0.386$, Fig. 2.9).

Space Pre-emption

The flies were not pre-empting space required by the weevil. The expected distribution of weevil eggs across the fly frequency distribution, assuming weevil oviposition was random, was not significantly different from the observed egg distribution ($\chi^2=0.085$, d.f.=7, $p>0.05$, Fig. 2.10). There was, however, significant differences between the expected and observed adult weevil emergence rates across the fly frequency distribution ($\chi^2=32.95$, d.f.=6, $p<0.05$, Fig. 2.11). Weevil emergence was significantly higher than expected if the head did not contain any galls, and significantly lower than expected if the head was attacked with two or more galls. There was no significant difference between observed and expected weevil emergence in heads containing one gall.

Differential Resource Acquisition

There was no significant difference between the proportion of spotted knapweed seed heads attacked by the fly at the point at which attack densities peaked, the week of August 11, 2002, compared to what was observed at the end of the field season, the week of October 13, 2002 ($\chi^2=1.081$, d.f.=1, $p=0.298$, Fig. 2.12). There was, however, a significant reduction in the proportion of heads attacked by the weevil from the point at which attack rates peaked, the week of July 28, 2002, compared to what was observed the week of October 13, 2002 ($\chi^2=18.789$, d.f.=5, $p=0.002$, Fig 2.13A). The proportion of seed heads in which the weevil was co-occurring with the fly decreased significantly over the course of the season ($\chi^2=26.275$, d.f.=5, $p<0.001$, 2.13B), with a corresponding increase in the proportion of seed heads in which the weevil had attacked on its' own ($\chi^2=26.275$, d.f.=5, $p<0.001$, Fig. 2.13C).

Weevils were most successful when a seed head was unoccupied by the fly and the proportion of seed heads from which an adult weevil emerged was negatively correlated with the number of fly galls present within the seed head (n=5, r=-0.984, p=0.003, Fig. 2.14).

Overgrowth Mechanism

The proportion of galls destroyed through contact with the weevil increased significantly over the course of the season ($\chi^2=72.890$, d.f.=5, p<0.001, Fig. 2.15), but this didn't appear to significantly reduce the fly population at the site (see Fig. 2.12). This increase in gall destruction did correspond with the phenological development of the weevil. Gall destruction increased as the proportion of late instar and pupae-stage weevils increased (see Table 2.1.)

Multivariable Hypothesis Testing

The probability of an adult weevil emerging from a spotted knapweed seed head is significantly affected by the relationship between peak weevil oviposition and the development stage of the seed head at the time of peak weevil oviposition, as well as by the mean number of fly galls in the seed head (Table 2.2.). The logistic regression model is:

$$P(Y_{\text{weevil}}=1) = 1 / 1 + \exp (-(-1.661 + 3.075X_{\text{pheno}} - 0.252X_{\text{fly}}))$$

where P is the probability of a seed head containing an emerged adult weevil, X_{pheno} = the proportion of total seed heads on the plant at the stage 4 development category, and X_{fly} = the mean number of gall flies per spotted knapweed seed head on the plant. The Wald statistic indicates a more important role for plant availability on weevil attack compared to fly density.

Discussion

In the natural world, observations of patterns of distribution and abundance are typically the result of several ecological processes operating on populations of organisms simultaneously (Quinn and Dunham 1983, Karban 1986, 1989). As such, one objective of ecological research should be to identify all of the factors that contribute to observed population patterns. Once identified, the second objective should be to quantify the relative contribution of each of those factors (Hassell 1985). The effects of host plant phenology and interference competition from the fly on weevil attack rates were modeled simultaneously. Although the species do compete, and although the presence of the fly significantly reduces weevil attack rates, it was host plant phenology that contributed most to weevil abundance (Table 2.2). Because no significantly negative effects of the weevil were detected on the fly in chapter 1, the equivalent model was not run for fly attack.

Substantial evidence has accumulated which demonstrates that variable plant phenology can have a significant influence on the density of insect herbivore populations (Wint 1983, Aurbach and Simberloff 1984, Fowler and Lawton 1984, Strong et al. 1984b, Crawley and Akhteruzzaman 1988, Raupp et al. 1988, Thompson 1988, Faeth 1990, 1991, Hunter 1990, 1992, Aurbach 1991, Watt and McFarlane 1991, Hunter and Lechowicz 1992, Hunter and Price 1992, Quiring 1993, 1994, Connor et al. 1994, Mopper and Simberloff 1995, Watt and Woidwod 1999, Hunter and Elkinton 2000).

The temporal aspects of resource development on plants are often dependent on environmental processes, such as temperature, and are therefore subject to variability and unpredictability. As such, an insect herbivores' 'window of opportunity' for resource

exploitation may likewise be ‘variable, narrow, and unpredictable’ (Hunter 1992, Hunter and Elkinton 2000). Insect populations that emerge either before or after the plant is at a developmental stage suitable for oviposition are not likely to achieve high attack densities, thereby reducing the likelihood that density-dependent processes like interspecific competition will be of much significance (Price 1997). Alternatively, when insect response is strongly correlated to host plant resource availability, populations may become eruptive, at which point density-dependent processes become more important. These dynamics are particularly relevant for gall forming insects, which tend to be highly specialized and attack the plant at a specific stage of development (Bernstein and Gobbel 1979, Weis et al. 1988). The distribution of the fly in this study illustrates how plant availability can influence attack densities, and subsequently interspecific competition with the weevil (See Fig.’s 2.3, 2.5, 2.14).

Variability in resource availability can also generate aggregated insect distributions, whereby the portion of resources that were available is heavily attacked (Inouye 1999), and interspecific competition becomes intensified. Both species, early in the season, occupied the same heads at the same time at a rate significantly higher than would be expected if insect distribution were random (Fig. 2.7). Plants with which the fly was well synchronized had experienced increasing rates of attack from the fly (Fig. 2.5). As fly densities increase, so do the frequency, intensity, and consequences of interspecific competition. Similar results have been observed with other insects (Fay and Whitham 1990, Quiring 1993).

Resource Pre-emption

Resource pre-emption is the most common competitive mechanism for animals in general (Schoener 1983), and has been documented previously for gall formers in particular (Karban and Myers 1989, Faeth 1992, Floate and De Clerck Floate 1993, Masters et al. 1993). As galling insects sequester nutrients, plant processes and functions relying on those nutrients become disrupted (Weis et al. 1988, Crawley 1989b, Williams and Witham 1988). The result of altered growth of some plant parts or processes could have consequences on other, later feeding species (Weis et al. 1988, Floate and De Clerck Floate 1993). Harris (1989) reported that spotted knapweed heads with high gall fly densities do not flower. Even though the results of this study did not find any evidence of disrupted flowering phenology (Fig. 2.8 and 2.9), the weekly evaluation may have been too coarse of a time-scale; high gall densities may have more subtle plant effects that are equally disruptive to weevil success. Gall forming species have been reported to reduce seed production as well as to delay seed ripening (Kirkland and Goeden 1978, Marshall et al. 1985, Trumble et al. 1985). If it occurred, such a delay could potentially increase mortality for newly hatched weevil larvae on knapweed, which begin to feed on immature seeds immediately upon emergence. The consequence to the weevil of a gall-induced delay in seed production would be starvation and would explain the high mortality rates observed for weevil larvae (Fig. 2.13). Additional support for this mechanism is provided by a comparison of Fig. 2.10 and Fig. 2.11. Weevil oviposition was independent of gall density, but weevil survival was significantly lower than expected in heads with more than one gall, suggesting resources required by the weevil were unavailable.

Space Pre-emption

Harris (1991) predicted for the knapweed system, that once the fly gall hardens, the seed head would be avoided by other species. The presence of so-called ‘differential oviposition behaviour’ may permit species to avoid interspecific competition within knapweed capitula (Stiling and Strong 1983, Briese 1996), as has been demonstrated in other systems (Karban and Myers 1989, Tomlin and Sears 1992), where species are able to discriminate between which plants had been attacked previously. Zwolfer (1988) noted that insect species with the strategy of feeding on maturing achenes in the flower head receptacle, like the weevil, are able to avoid heads already occupied by another species. Zwolfer (1988) cited *Larinus sturnus* in *Centaurea scabiosa*, avoiding heads already attacked by an early-season tephritid.

In the present study, weevil oviposition, as determined by egg distribution, was independent of gall fly density (Fig. 2.10). The weevil did not discriminate between a seed head that had already been attacked by the fly and one that hadn't. It appears as though weevil oviposition is more a function of host plant suitability, rather than the presence or absence of the fly (Ofomata et al. 1999). The evidence does not support the space pre-emption mechanism of competition.

Differential Resource Acquisition

Although observed patterns of weevil mortality provide indirect support for this mechanism, a more appropriate measure would have been weevil body mass in seed heads occupied by both species. The difficulty associated with this is that the two species rarely co-occur in the seed head beyond early instar stages of development. Additionally, because the fly and weevil are not found in the same head until after the fly has initiated

its gall, its unlikely that there is a simultaneous utilization of resources, a requirement of this mechanism. Rather, resources have likely been rendered unavailable by the fly, well before the weevil attacks the seed head. So even though weevil mortality increased in heads also attacked by the fly, the observed patterns are better described by resource preemption.

Overgrowth

This mechanism describes very accurately the effect the weevil is having on the fly. The observed increase in destroyed galls corresponds with weevil pupation and the construction of a pupal chamber (Fig. 2.15, Table 2.1). Portions of destroyed galls were commonly found in pupal cell aggregate, and occasionally the fly larva was displaced from the gall, but still intact. This particular mechanism has been commonly documented for plants, but never for animals (Schoener 1983).

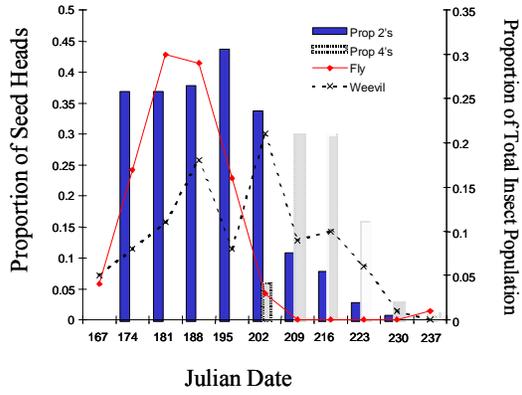
Another important implication of this overgrowth effect of the weevil on the fly is that the outcome of the competitive interaction changes over the course of the season. The fly 'wins' early in the season, but if the weevil is able to survive in a seed head also attacked by the fly, it seems to displace the fly during the construction of its pupal chamber. This seasonal reversal in competitive dominance has also been reported for spiders (Spiller 1984).

The fly on spotted knapweed illustrates how a biological control agent can attain high densities when released from its natural enemies, and when attack is synchronized with plant phenology. In this case, high densities of the insect herbivore has not translated into measured control of the weed population. Instead, the fly has interfered

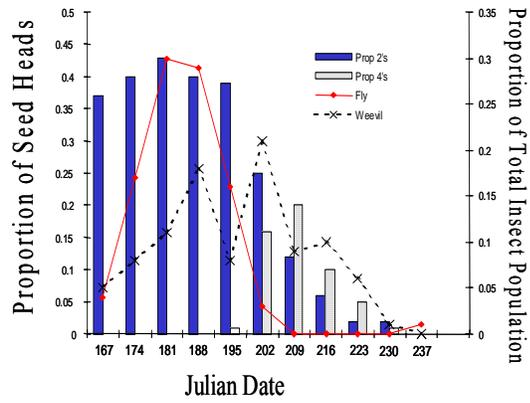
with a more recently introduced, more effective biological control agent. The mechanism of that interference is best described by resource preemption.

Chapter 2 Figures

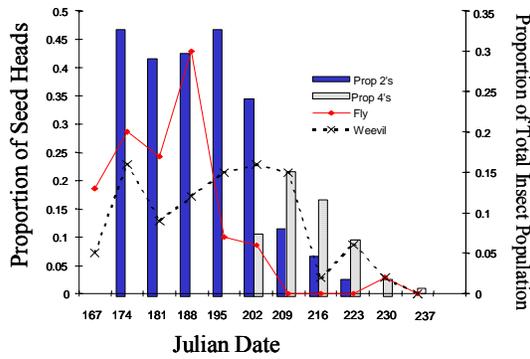
A) Site 1



B) Site 2



C) Site 3



D) Site 4

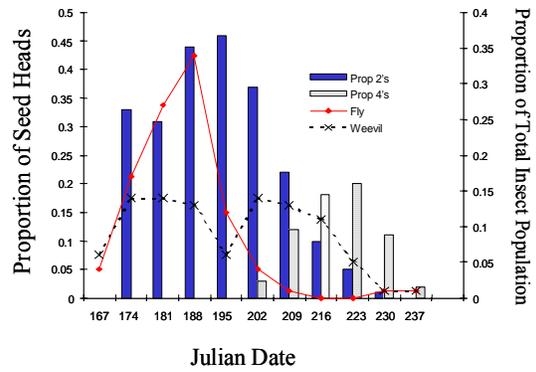


Figure 2.1. Adult recruitment curves for the weevil and the fly plotted with the spotted knapweed development of stage 2 and stage 4 seed heads from the week of June 16, 2002 to the week of August 25, 2002. The four monitoring sites were: 1) Heinz Cabin, 2) Seven-Mile Reservoir, 3) Brilliant, and 4) Sanca Creek.

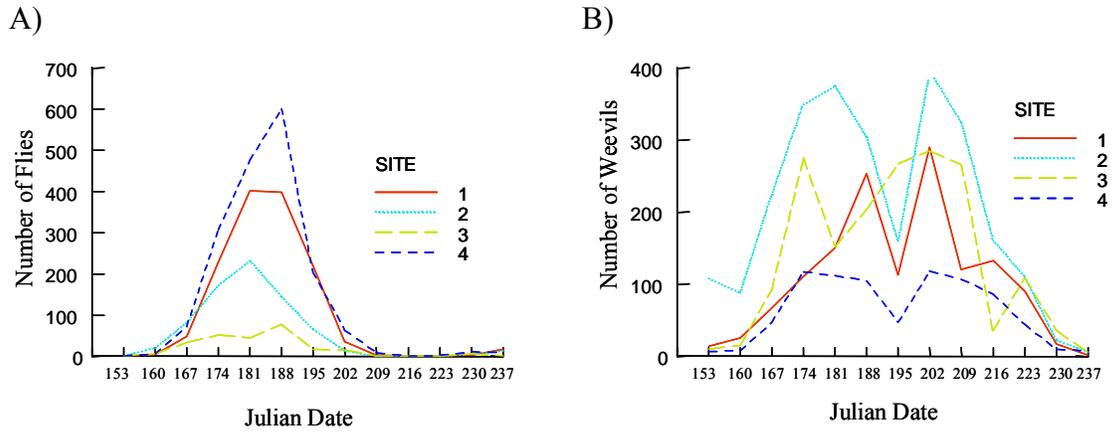


Figure 2.2. Adult recruitment densities for **a)** the fly and **b)** the weevil at weekly intervals at each of the four monitoring sites during the 2002 field season. Note the difference in scale on the y-axis.

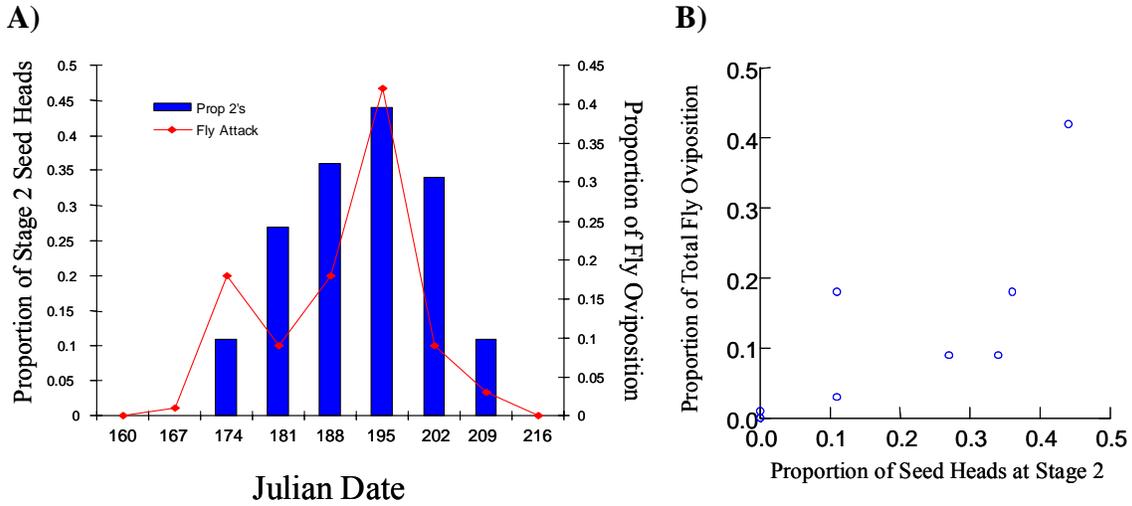


Figure 2.3. **A)** The proportion of spotted knapweed seed heads at the development stage required for oviposition by the fly and the proportion of total fly oviposition that occurred between June 9 and Aug. 4, 2002 at Heinz Cabin 2002. **B)** The correlation between the proportion of seed heads at stage two on a particular week and the proportion of seed heads attacked by the fly on that week, from June 9 to Aug. 4, 2002 ($n=9$, $r=0.778$, $d.f.=1$, $p=0.014$).

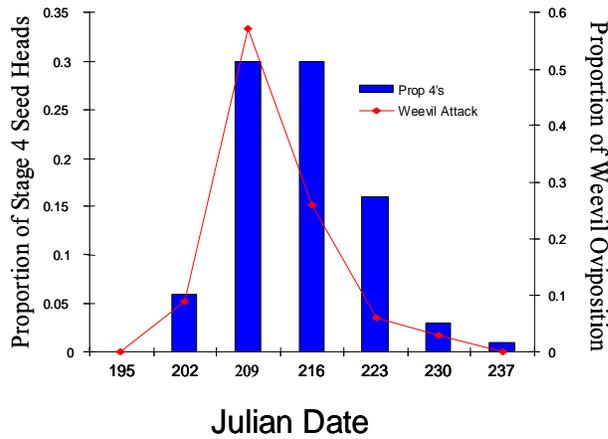
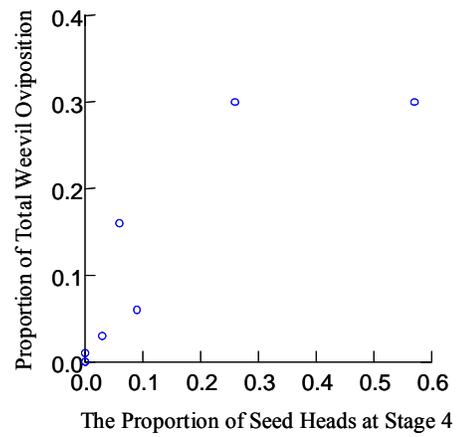
A)**B)**

Figure 2.4. **A)** The proportion of spotted knapweed seed heads at the development stage required for oviposition by the weevil and the proportion of total weevil oviposition that occurred between July 14 and Aug. 25, 2002 at Heinz Cabin 2002. **B)** The correlation between the proportion of seed heads at stage four on a particular week and the proportion of seed heads attacked by the weevil on that week, from July 4 to Aug. 25, 2002 ($n=9$, $r=0.778$, $d.f.=1$, $p=0.014$). ($n=8$, $r=0.865$, $d.f.=1$, $p=0.006$).

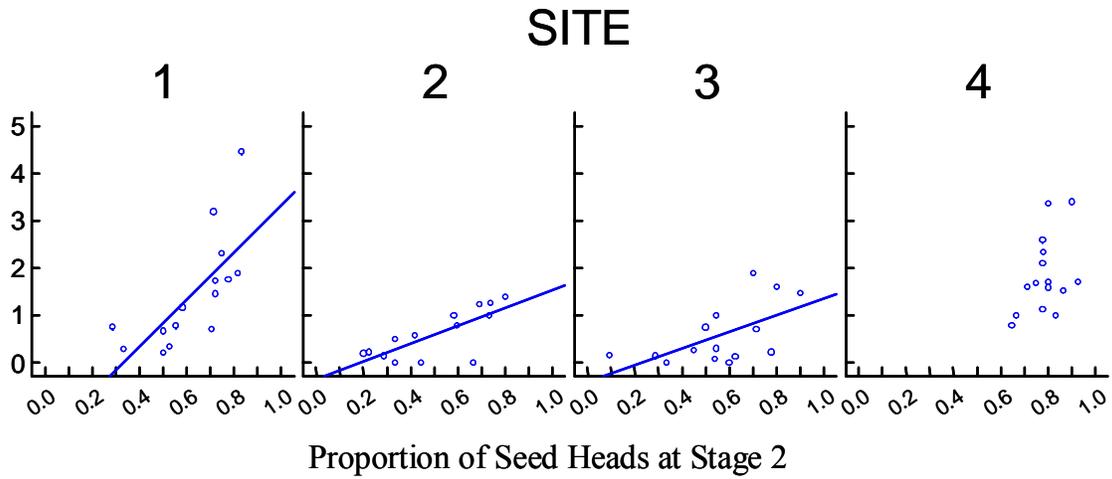


Figure 2.5. The relationship between the proportion of heads at stage 2 on 15 spotted knapweed plants/site at the time of peak oviposition by the fly and the mean number of flies per head on those plants at the end of the 2002 field season. Proportions were arcsin square root transformed for the correlation analysis. (Site 1: $r=0.723$, $p=0.002$; Site 2: $r=0.748$, $p=0.002$; Site 3: $r=0.583$, $p=0.023$; Site 4: $r=0.412$, $p=0.127$).

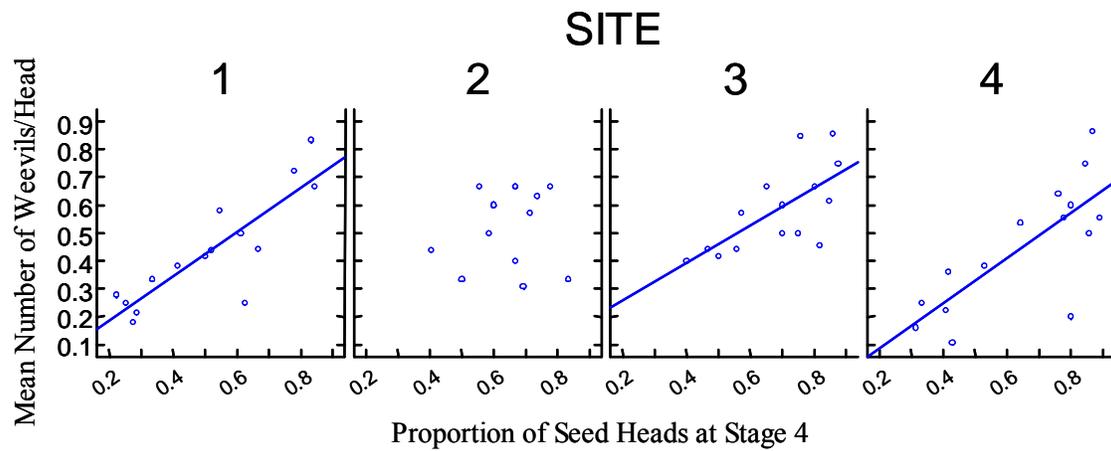


Figure 2.6. The relationship between the proportion of seed heads at stage 4 on 15 spotted knapweed plants/site at the time of peak oviposition by the weevil and the mean number of weevils per head on those plants at the end of the 2002 field season. Proportions were arcsin square root transformed for the correlation analysis. (Site 1: $r=0.859$, $p<0.001$; Site 2: $r=0.077$, $p=0.795$; Site 3: $r=0.671$, $p=0.006$; Site 4: $r=0.758$, $p=0.001$).

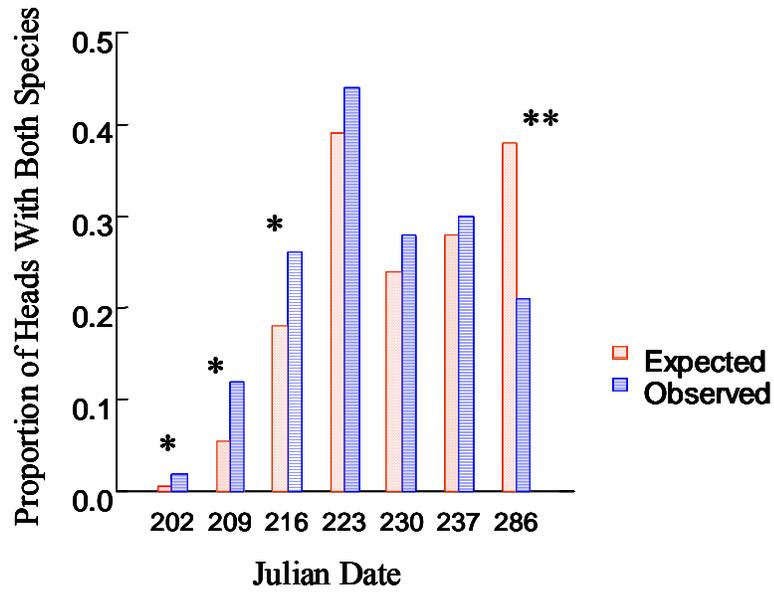


Figure 2.7. The expected and observed proportions of spotted knapweed seed heads attacked by both the fly and the weevil over the course of the field season at Heinz Cabin 2002. * Indicates a significant difference between the expected and observed value as measured by the Chi-square statistic for Goodness-of-fit at $\alpha=0.05$. ** The significance of the chi-square statistic at week 20 was $0.1 > p > 0.05$. JD 202 = July 21, 2002 and JD 286 = October 13, 2002.

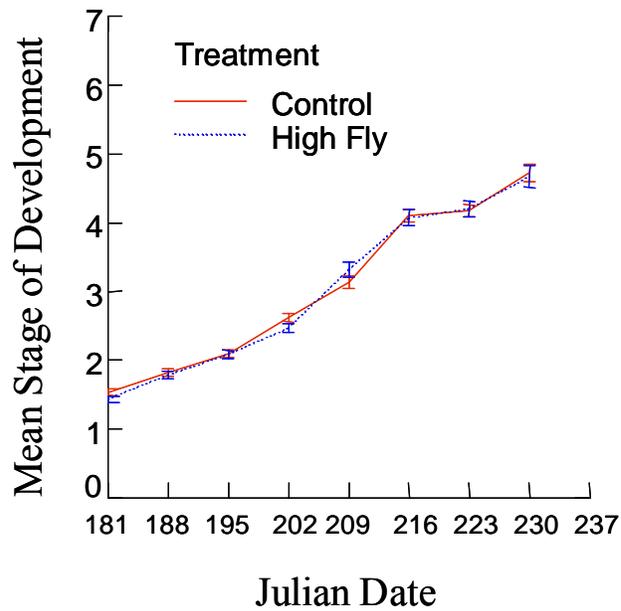


Figure 2.8. The mean (\pm SEM) stage of spotted knapweed seed head development in both the control treatment and the high fly treatment plotted over the field season at Rabbit Ridge 2002. Means were compared with a 2-way ANOVA with treatment, date, and treatment*date as factors but the interaction term was not significant ($F=0.624$, $d.f.=7,464$, $p=0.736$). JD 181 = June 30, 2002 and JD 237 = August 25, 2002.

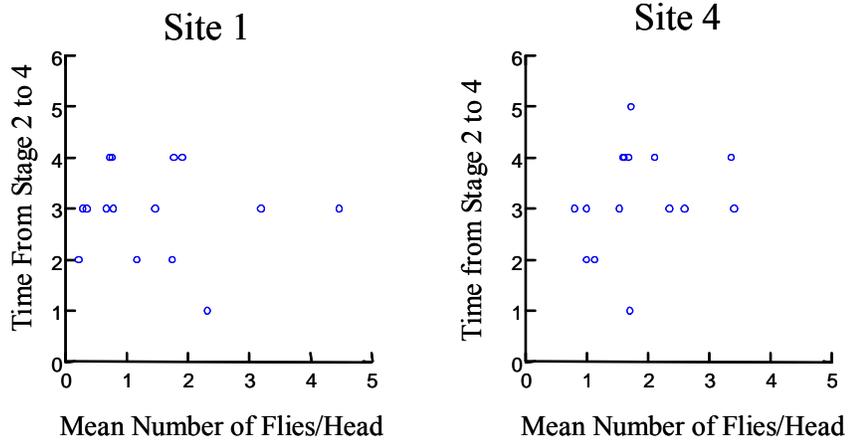


Figure 2.9. The relationship between the time (in weeks) from the peak of development stage 2 and the peak of development stage 4 on 15 spotted knapweed plants per site and the mean number of flies per head on those plants at the end of the season (Site 1: $r=0.077$, $p=0.781$; Site 4: $r=0.241$, $p=0.386$).

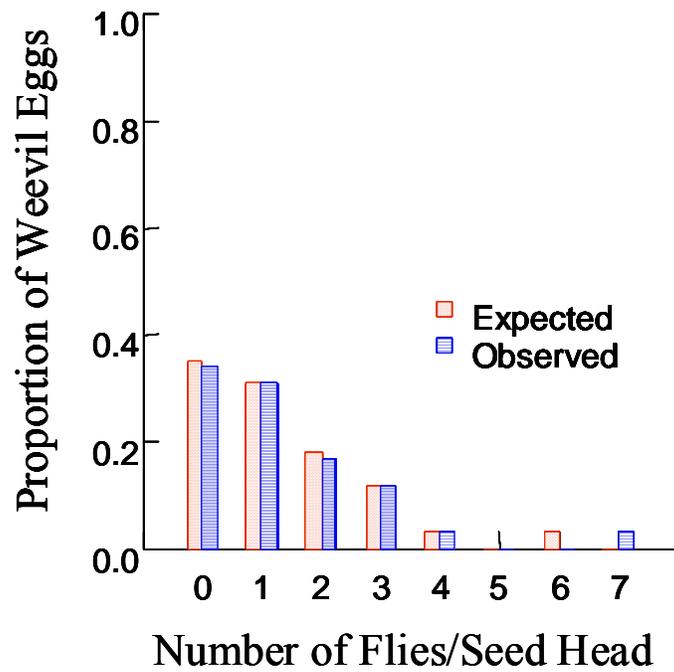


Figure 2.10. The expected and observed proportions of total weevil eggs in spotted knapweed seed heads across the fly frequency distribution at Heinz Cabin 2002 ($n=35$, $\chi^2=0.085$, $d.f.=7$, $p>0.05$). * Indicates a significant difference between expected and observed values.

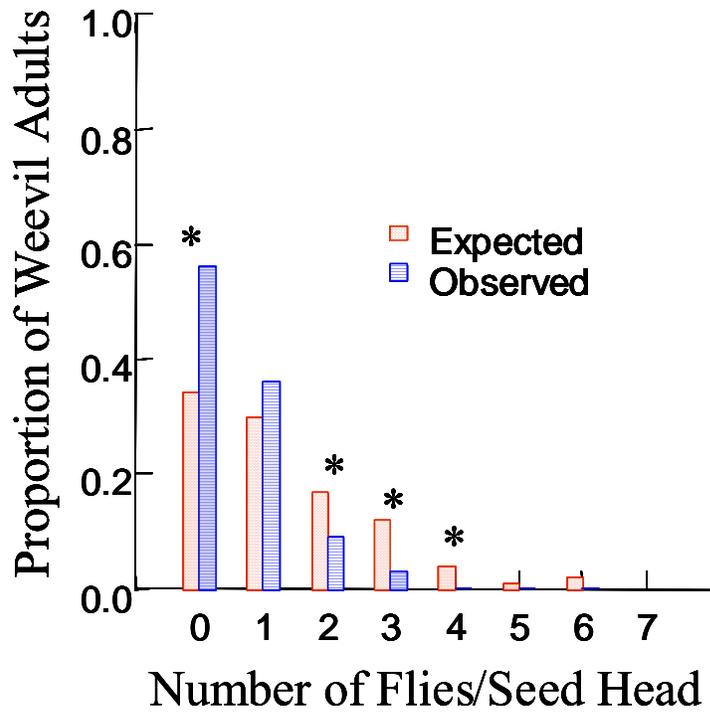


Figure 2.11. The expected and observed proportions of total emerged weevils in spotted knapweed seed heads across the fly frequency distribution at Heinz Cabin 2002 (n=138, $\chi^2=32.95$, d.f.=6, $p<0.05$). * Indicates a significant difference between expected and observed values.

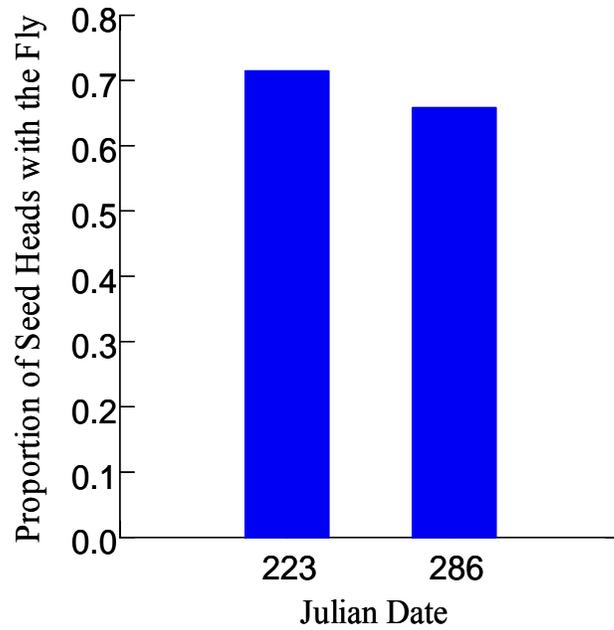


Figure 2.12. The difference between the proportion of spotted knapweed seed heads attacked by the fly when the population peaked and the proportion attacked at the end of the field season at Heinz Cabin 2002 ($\chi^2=1.081$, d.f.=1, $p=0.298$). JD 223 = August 11, 2002 and JD 286 = October 13, 2002.

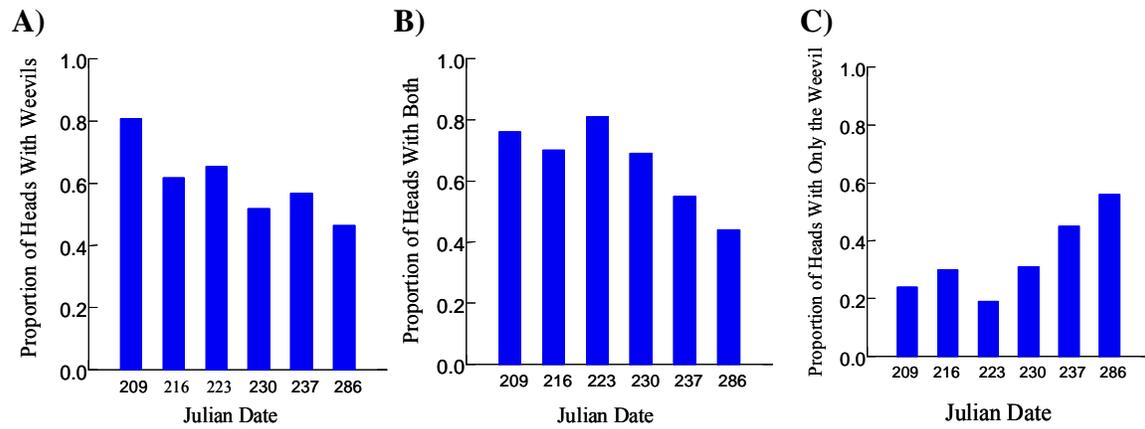


Figure 2.13. The change in the proportion of spotted knapweed seed heads (n=641) attacked by **A)** the weevil in all heads dissected ($\chi^2=18.789$, d.f.=5, $p=0.002$), **B)** the weevil in heads with the fly ($\chi^2=26.275$, d.f.=5, $p<0.001$), and **C)** the weevil in heads where it occurred on it's own ($\chi^2=26.275$, d.f.=5, $p<0.001$) from the point of peak attack through to the end of the field season at Heinz Cabin 2002.

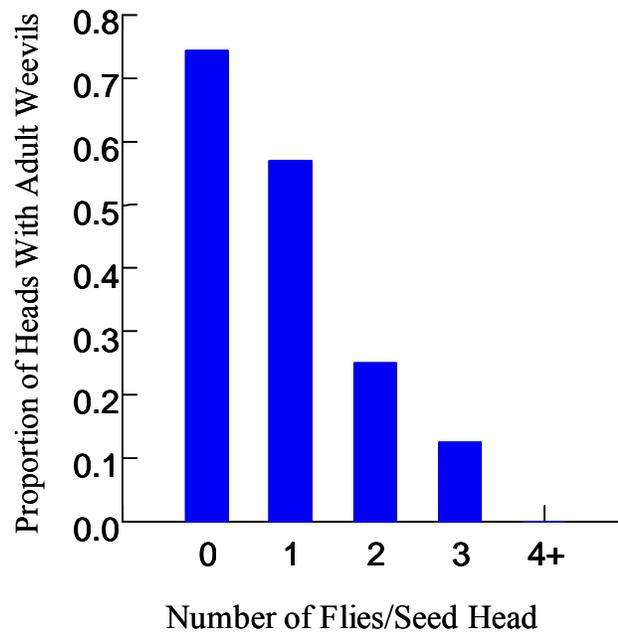


Figure 2.14. The relationship between the proportion of spotted knapweed seed heads attacked by the weevil and the fly frequency distribution at Heinz Cabin 2002 (n=5, $r=-0.984$, $p=0.003$).

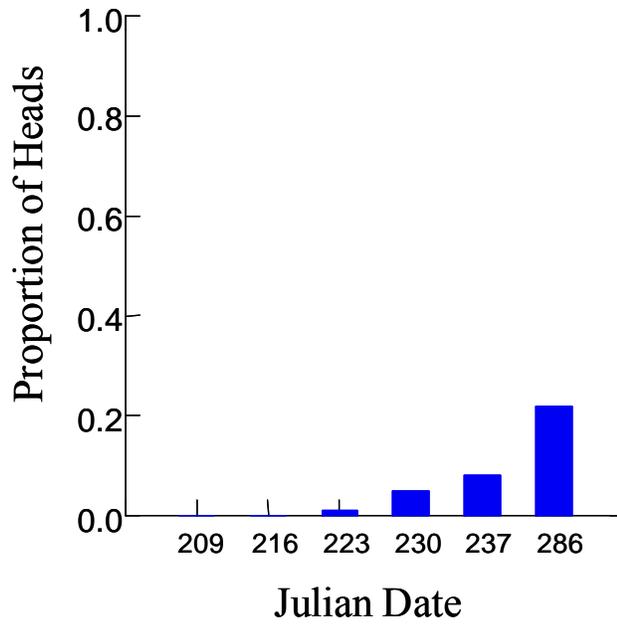


Figure 2.15. The proportion of spotted knapweed seed heads containing destroyed fly galls and weevil larvae/pupae over the field season at Heinz Cabin 2002 ($\chi^2=72.890$, d.f.=5, $p<0.001$). JD 209 = July 28, 2002 and JD 286 = October 13, 2002.

Table 2.1. The phenological development of weevils in spotted knapweed seed heads from egg, to larval, to pupal stages, expressed as proportions of the total number of weevils counted, over the field season at Heinz Cabin 2002.

Julian Date	n Weevils	Prop. Eggs	Prop. Larvae	Prop. Pupae	Total
202	6	1	0	0	1
209	52	0.83	0.07	0	1
216	100	0.19	0.8	0.01	1
223	92	0.02	0.96	0.02	1
230	95	0.01	0.83	0.16	1
237	134	0	0.59	0.41	1

Table 2.2. The results of a logistic regression analysis of the effect of host plant phenology and the mean number of fly galls on the two-state probability of an adult weevil emerging from a spotted knapweed seed head.

Parameter	B	SE	Wald	d.f.	Sig.
Prop. Stage 4 Seed Heads	3.075	0.303	102.925	1	<0.001
Fly Mean	-0.275	0.065	14.926	1	<0.001
Constant	-1.661	0.211	62.009	1	<0.001

General Discussion

Insect establishment and population growth are fundamental aspects of an effective weed biocontrol program. Understanding the mechanisms that either promote or inhibit population growth is a critical component of a successful program. It is commonly accepted that multiple processes operate within communities simultaneously, each at varying intensities and each with varying consequences for the distribution and abundance of organisms comprising those communities. The challenge to ecologists is both to identify the important processes and quantify their role in structuring communities.

Denno et al. (1995) found competition in 76% of the 193 pair-wise interactions they reviewed, and other recent reviews also conclude that competition can be an important ecological process operating in insect communities (Faeth 1987, Stewart 1996, Reiz and Trumble 2002). Interspecific competition appears to be a central process in the population dynamics of both the fly and the weevil on spotted knapweed. The fly interfered with the weevil such that the population of the weevil was significantly reduced compared to situations in which the weevil was attacking on its own. This pattern was consistent across both years of experimentation, at all sites, and in all experiments.

The consequence of competition is reduced control of spotted knapweed seed when both species are present at a site compared to what would occur if the weevil were released on its own. These results on spotted knapweed support arguments to release the single-most effective species rather than multiple species in biological control protocols. This application is limited, however, to the most effective insect within a particular

feeding guild. There is evidence for positive species associations on knapweed between feeding guilds (Bourchier and Crowe, unpublished data). The implications for biological control protocol are to ensure, prior to release, that the biocontrol agent is actually capable of having an impact on the population dynamics of the target host.

In addition to the potential for antagonistic interactions between insects in multi-agent systems, redundancy in agent releases may have financial and ecological costs as well. A biological control program is a substantial financial investment (Harris 1979, 1990, Kareiva 1996, McFadyen 1998) with little return when agents fail to suppress the weed population. Additionally, there are costs associated with non-target ecological effects and the disruption of ecosystem processes that may accompany the introduction of exotic species (Howarth 1991, Kareiva 1996, Simberloff and Stiling 1996, Thomas and Willis 1998). These problems could be minimized if there were 'fewer releases of agents not likely to suppress pest populations, and fewer releases of insects that will interact antagonistically' (McEvoy and Coombs 1999). For this reason, Louda et al. (2003) has recommended collecting additional information on population parameters at the pre-release stages to better predict things like the impact of the insect on the host plant population, insect establishment and population growth, interactions with other biocontrol agents, and non-target effects.

Fly and weevil attack rates were highly dependent on spotted knapweed phenology. Populations of both insect species achieved high densities on plants with which they were closely synchronized. However, attack rates of both species were significantly reduced as asynchronization with individual plants increased.

Interspecific competition is a density dependent process. In circumstances in which both species are well synchronized with host-plant development, outbreak densities are likely to occur, and the frequency and intensity of interspecific interactions will increase. Spotted knapweed seed heads that were phenologically available to ovipositing individuals of both species had a significantly higher than expected co-occurrence distribution pattern early on in the season.

Specific plant-mediated mechanisms of interspecific competition were also studied in order to precisely describe the type of competition between the fly and the weevil. The mechanism best describing the effect of the fly on the weevil was resource preemption, with no evidence supporting differential resource acquisition and space preemption mechanisms, and although the overgrowth mechanism was observed, its effects were not significant.

The weevil is asynchronized with a proportion of seed heads, resulting in a restriction on resource availability and a limitation placed on population size. Couple this with larval interference from the fly and the weevil population is suppressed below outbreak densities, which has serious implications on the effectiveness of the weevil as a biocontrol agent. Although the weevil has the potential to significantly reduce the reproductive capacity of spotted knapweed, observed seed reductions are less than what is theoretically possible because of suppressed attack rates.

Although competitive interactions between the fly and the weevil had a significant, negative effect on the weevil population, the density independent processes associated with interactions between the host plant and the insect herbivores played more of a central role in the short-term populations of both the fly and the weevil. It is likely

that host plant dynamics is the more important and consistent mechanism regulating the populations of both insect species, and that competition, while occasionally intense, is variable and dependent host plant dynamics.

It should also be noted that the focal scale of this research was at the seed head level, and that other processes are likely operating at broader scales which permit both species to co-occur at a particular site and have enabled weevil densities to increase consistently over time at sites where the weevil has been released.

Future Research

These experiments have focused on larval interactions between the fly and the weevil and may have overlooked some of the other possible interactions occurring at the adult stages. Possible agonistic interactions at the adult stage may favor the weevil, and would help to explain the ability of both insect species to co-exist at the patch scale. They may also have important implications for biocontrol protocol in terms of the best time to release the weevil for optimum establishment rates and population growth rates.

Plant architecture, the distribution of aboveground biomass in space, may be another important bottom-up influence on insect herbivore attack rates. The effect of architecture on insect distributions and subsequent interspecific interactions is presently being investigated. Two lines of inquiry serve as the basis for this research: first, the specific plant attributes and how they interact with both insect species, and second, how early-season feeding-induced alteration of architecture by the weevil affects the fly and its ability to exploit the host plant.

Harris (1980) reported that knapweed flower heads producing a large number of seeds would be the preferred resource, and would be selected more frequently by ovipositing female flies. Aggregation by insect herbivores onto a particular portion of a resource through preferential resource selection mechanisms is well documented (McClure 1980). The aggregated distribution observed at the beginning of the season in the present study may be a function of increased resource quality. This type of response by insect herbivores to host plant resources has been referred to by Price (1997) as the plant vigour hypothesis, and is a concept that merits further investigation on its applicability within this system.

Finally, additional research is required to verify the hypothesis posed here that attack by the gall fly delays the temporal seed production dynamic such that immature seeds are not available to the emerging weevil larvae, thereby causing high rates of mortality in seed heads in which the two species co-occur early in the season.

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