

**IMPACT AND BIOLOGICAL CONTROL OF *LYTHRUM*  
*SALICARIA* IN SOUTHWESTERN BRITISH COLUMBIA**

by

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## ABSTRACT

Purple loosestrife, *Lythrum salicaria*, is a Eurasian plant that has invaded North American wetlands over the last 200 years. As a precautionary measure, but without detailed knowledge of the ecological impact of *Lythrum* on native species, a biological control project was started in the 1990s with the introduction of *Galerucella californiensis*, a leaf-feeding beetle of European origin.

To measure the ecological impact of *Lythrum*, I assessed the effect of *Lythrum* on a rare, native plant species, *Sidalcea hendersonii*. To evaluate the biological success of the biological control project, I investigated the effectiveness of the biological control agent and identified factors associated with among-site variability in defoliation levels.

*Lythrum's* impact on *Sidalcea* was not stronger than the impact of native species, possibly owing to the early emergence in the spring and the tall growth of *Sidalcea*. The abundance of *Lythrum* in a marsh where it co-occurs with *Sidalcea* did not increase significantly from 1979 to 1999, which suggests that the invasion process was largely completed by 1979.

The effectiveness of the control agent was dependent on site condition. In tidal areas, the beetles could not establish due to the tidal disturbances. At sites where the control agent established, the reproductive performance and dry biomass of *Lythrum* were strongly reduced by beetle feeding damage in the same year, but stem density only declined at one site, Chilliwack, after severe herbivory over a four-year period. At most other non-tidal sites, plant stem

density increased, and stem density changes were inversely related to the average beetle feeding damage during the previous four years.

Beetle feeding damage was associated with predation on *G. californiensis* eggs, but not predation on any other life-history stage, or plant quality. The densities of crawling, invertebrate predators were not generally related to predation levels, suggesting that predation did not vary in response to predator abundance, but to predator attack levels.

I discuss the level of success of this biological control project and the need for control methods in tidal areas.

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# CHAPTER ONE

## INTRODUCTION

In natural ecosystems, exotic species may cause irreversible damage or destruction (McFadyen 1998 and references therein, Myers and Bazely 2003), and are considered one of the leading causes of human-induced extinctions of species (Diamond 1989). The use of mechanical and cultural methods and herbicide treatments to control exotic weeds are often unacceptable or not feasible in natural ecosystems (McFadyen 1998, Fowler et al. 2000), and with few or no other available options for effective weed management, classical biological control (biocontrol) is considered a last resort (Fowler et al. 2000). Biocontrol is the deliberate introduction of specialist, exotic species (control agents), such as parasitoids, and herbivores or diseases, to reduce the abundance of pest species. Biocontrol is a sustainable but irreversible method: once a control agent is widely established, further releases are not necessary, but the species is likely to persist and cannot be eradicated. In the last 100 years, nearly 400 species of invertebrates and fungi have been released against 133 weeds worldwide (Fowler et al. 2000).

Some biological control programs have been spectacularly successful; an example is the control of prickly pear cactus (*Opuntia* spp.) in Australia (Julien and Griffiths 1998, Myers and Bazely 2003). However, the average success rate of biological control projects varies with the region and the definition of success. Estimates range from below 20 % to over 80 % (Hall and Ehler 1979, Julien 1989, McFadyen 1998, Fowler et al. 2000, Myers and Bazely 2003).

Recent observations that introduced biological control agents can have dramatic negative effects on non target species (Howarth 1991, Louda et al. 1997, Louda et al. 2003a) has led to wide-spread concern over the use of this technique. A total of twelve biocontrol agents against weeds have been reported to attack non target plants; two of them may have a serious impact on the survival of rare native species (Louda et al. 1997, McFadyen 1998, Fowler et al. 2000, but see Stiling et al. 2000). Given the irreversibility of biocontrol, the environmental benefits and risks of a biological control program need to be evaluated carefully before releases are made (McFadyen 1998, Louda et al. 2003a, Louda et al. 2003b). The recent biological control program against *Lythrum salicaria* L., purple loosestrife, has incited considerable debate over the way in which the benefits of control agent introductions were evaluated prior to the onset of the control program.

*Lythrum* is a perennial plant of Eurasian origin, which was first introduced to North America in the early 1800's (Mal et al. 1992). Introductions were accidental via the ballast of ships and in sheep wool, as well as deliberate as a medicinal plant, a source of nectar and pollen for bees and as an ornamental plant (Mal et al. 1992). Since the plant has spread considerably since its introduction and now occupies an estimated 120,000 ha of North American wetlands (Farnsworth and Ellis 2001), it received considerable attention as a biological control target (Hight and Drea 1991, Malecki et al. 1993).

*Lythrum* has been declared a noxious weed in Manitoba and Alberta and in several American states (Mal et al. 1992, Royer and Dickinson 1999), and a biological control project was initiated in 1993 with the release of up to four insect herbivore species in many locations across North America (Hight et al. 1995). Early reports from the biocontrol program in the United States of America (listed in Julien and Griffiths

1998) suggested that this control agent was able to reduce the abundance of the weed. Negative effects on non target species appear negligible as determined by field monitoring (Blossey et al. 2001a, Corrigan et al. 1998).

This program was recently criticized on the grounds that the detrimental impact of the weed on native communities was not sufficiently demonstrated before the onset of the project. It was argued that the evidence for a detrimental impact of *Lythrum* on native species and communities is largely anecdotal and that rigorous, scientific testing to determine the actual impact of *Lythrum* was not done (Anderson 1995, Hager and McCoy 1998, but see Blossey et al. 2001b). It is possible the obvious appearance of *Lythrum* due to its bright purple flowers led to an overestimate of its abundance and its impact (Booth et al. 2003). Given this controversy and the fact that many successful biological control programs have resulted from the introduction of a single control agent (Denoth et al. 2002), only one species, the leaf-feeding beetle *Galerucella californiensis* L. has been widely released in British Columbia since 1993.

The purpose of this study is twofold. First, as a measure of the detrimental impact of *Lythrum*, and thus the potential benefits of successful biocontrol, I determine the influence of *Lythrum* on *Sidalcea hendersonii* S. Wats (Henderson's checker-mallow), a rare native species of coastal British Columbia (Chapter 2). Second, I investigate the potential of *G. californiensis* as a successful control agent of *Lythrum* in southwestern British Columbia by determining the spatial and temporal variability in beetle feeding damage, by identifying factors associated with varying feeding damage among sites and by assessing the effect of control agent feeding on *Lythrum* performance (Chapter 3).

These studies have been undertaken with the aim of making the "art" of biological control a more predictive "science".

## CHAPTER TWO

# INTERACTION OF THE INVADER PLANT *LYTHRUM SALICARIA* WITH THE RARE, NATIVE PLANT *SIDALCEA HENDERSONII*

### **1. Introduction**

Biological invasions cause global problems of growing ecological and economic relevance (Vitousek et al. 1996, Wilcove et al. 1998, Mack et al. 2000, Pimentel et al. 2000). A well-known example of an introduced species is *Lythrum salicaria* L., purple loosestrife, a Eurasian plant that has invaded North American wetlands over the last two hundred years. The rapid spread and high abundance of *Lythrum* in invaded areas has led to concerns that the plant may outcompete native plant species and degrade the value of infested areas for wildlife. Thompson et al. (1987) report that *Lythrum* is able to form monospecific stands within 1 - 2 years, and that these stands can maintain themselves for more than 20 years. The persistence of established stands might be explained by the strong competitive ability of *Lythrum* due to its high total biomass, tall plant size and fast growth (Gaudet and Keddy 1988).

However, it has been argued that scientific evidence from field experiments confirming the detrimental effect of *Lythrum* on native species and communities is lacking, inconclusive or contradictory (Anderson 1995, Hager and McCoy 1998). A growing number of studies address the question of the ecological effects of *Lythrum*, yet, the results of these studies are equivocal. Negative effects (see review in Blossey et al. 2001b) have been reported on native plant pollination and seed set (Grabas and

Lavery 1999, Brown and Mitchell 2001, Brown et al. 2002), native plant biomass (Gaudet and Keddy 1988, Keddy et al. 1998, Keddy et al. 2000), bird biodiversity (Whitt et al. 1999) and nutrient cycling (Grout et al. 1997). However, other studies detected no evidence for detrimental effects on plant diversity (Anderson 1991, Treberg and Husband 1999), invertebrate communities (Gardner et al. 2001), microbial processes (Otto et al. 1999), or decomposition patterns (Welsch and Yavitt 2003). Farnsworth and Ellis (2001) suggest that the inconsistent results may be partly due to the difference in methods used to estimate *Lythrum* abundance. A more comprehensive understanding of the ecological effects of *Lythrum* on native species, communities and ecological processes can only result from observational and experimental studies of specific interactions.

Among the areas invaded by *Lythrum* in British Columbia's Lower Mainland is the tidal Ladner Marsh in Delta. Here, a rare, native plant species, Henderson's checker-mallow, *Sidalcea hendersonii* S. Wats. (Malvaceae) occurs. *Sidalcea* is an endemic species of coastal British Columbia, Washington and Oregon (Douglas et al. 1998), with only 50 to 60 scattered populations remaining (Love 2003). Thus, this species would appear to be a likely candidate of a plant that may be threatened by the invasion of a strong competitor.

I present the results of a study aimed at determining the impact of *Lythrum* on *Sidalcea*. *Sidalcea* was chosen as the target species for several reasons: i) as a rare and endemic species, *Sidalcea* is of particular concern; ii) data from a previous study (Bradfield and Porter 1982) allowed a comparison of the distribution and abundance of the invader and the native species in 1979 and 1999; iii) since both *Lythrum* and *Sidalcea* are tall-growing, herbaceous plants that co-occur in Ladner marsh, competition seems likely; iv) *Sidalcea* occurs in tidal areas for which no sustained means of

controlling the invader plant exist to date. If this study indicates that the impact of *Lythrum* on the rare native plant is stronger than the impact of native, co-occurring species, a need for efficient control methods for *Lythrum* in tidal areas could be demonstrated.

Specifically, I address the following questions:

**Question 1: Has *Lythrum* abundance increased in Ladner marsh between 1979 and 1999 and is a decrease of *Sidalcea* associated with it?**

**Question 2: Is there a negative relationship between *Lythrum* and *Sidalcea* in Ladner marsh?**

**Question 3: Is the competitive effect ( i.e. the ability to suppress other species *sensu* Goldberg and Werner 1983) of *Lythrum* on *Sidalcea* stronger than the effect of common native species?**

**Question 4: Is there evidence for a spatial or temporal segregation of *Lythrum* and *Sidalcea* that could mitigate competitive effects?**

## **2. Methods**

### **2.1 Study plants**

#### *Lythrum salicaria*

*Lythrum* is a perennial plant of Eurasian origin that was introduced to North America in the early 1800s and was first recorded in British Columbia as a herbarium specimen, collected in 1897. However, it did not persist, and was not reestablished until the 1920's (F. Ganders, University of British Columbia, pers. com.). The biology, ecology, history of invasion into North America and the environmental impacts of *Lythrum* have been described extensively elsewhere (Shamsi and Whitehead 1974, Thompson 1987, Gaudet and Keddy 1988, Anderson 1991, Mal et al. 1992, Keddy et al. 2000, Blossey et al. 2001b). Key characteristics of the plant are: (1) it grows in a wide range of environmental conditions (Mal et al. 1992 and references therein); (2) it grows rapidly and up to 2.7 m in height (Mal et al. 1992) with a high biomass, conferring a strong competitive ability (Gaudet and Keddy 1988); (3) individual plants are long-lived (> 20 years) and have a high survival once they are more than two years old (Anderson 1991); (4) seed production is high (estimates range from 100,000 –2,700 000 per plant and year) and the seeds form an abundant seed bank; the small and light seeds are easily dispersed by water, wind and animals (Mal et al. 1992 and references therein); (5) *Lythrum* successfully colonizes open soils, responding positively to many kinds of human and natural disturbances. Given the tendency of *Lythrum* to occupy sensitive, wet habitats such as stream banks, lakes and ditches, Hight and Drea (1991) argued that biological control is the most suitable and environmentally safe technique for managing this exotic plant.

## *Sidalcea hendersonii*

*Sidalcea hendersonii* S. Wats. (Malvaceae), Henderson's checker-mallow, occurs in a narrow geographical and ecological range: it is native to wet meadows and tidal marshes from southwestern British Columbia to northwestern Oregon. In Oregon, the species was found at only three locations in the last 40 years (Love 2003). In British Columbia, perhaps fewer than 30 populations of *Sidalcea hendersonii* exist (F. Ganders, University of British Columbia, pers. comm.), some consisting of a relatively large number of individuals. Human encroachment into wetlands and displacement by *Lythrum* are thought to contribute to the species' rarity (Douglas et al. 1998, Marshall 1998, Marshall and Ganders 2001). The plants can grow conspicuously tall, reaching 1.5 m in height, and can produce multiple stems with inflorescences. In Ladner marsh foliage is produced early in the growing season before many other species begin to grow (personal observations).

## **2.2 Study site**

All experiments were carried out at Ladner marsh, a tidal, mostly freshwater marsh, located along the Fraser River in Delta in British Columbia's Lower Mainland (49°6' N, 123°5' W). The marsh is species-rich, having a total of 57 vascular plant species (unpublished data). Bradfield and Porter (1982) detail the environmental conditions and the composition of the plant communities in the marsh. In some areas, particularly where the soil is waterlogged, the vegetation is dense and consists of *Lythrum*, intermixed with other nonnative and native plants. *Sidalcea* occurs frequently

throughout the marsh, particularly along drainage canals, but is only rarely present in poorly drained areas.

## 2.3 Experiments

### *Changes in species densities from 1979 to 1999*

Following Bradfield and Porter's methodology (1982), I conducted a study in Ladner Marsh in July and August of 1999 to record *Lythrum* and *Sidalcea* densities. Bradfield and Porter (1982) recorded all plant species and their ground cover in 108 1 m<sup>2</sup> quadrats in Ladner marsh in June and July of 1979. The quadrats were arranged in seven transects laid in a systematic fashion throughout the marsh. I determined the approximate transect locations and recorded the presence and ground cover of *Lythrum* and *Sidalcea* in 100 1 m<sup>2</sup> quadrats. Due to the creation of channels in the 1990s eight of the quadrats could not be repeated and were omitted from the original data to facilitate comparison. Since 20 years had elapsed between the two surveys, and the locations of the plots were not exactly the same, I assumed that the two samples were sufficiently independent for the purpose of statistical analysis. *Lythrum* and *Sidalcea* frequencies and ground cover were compared between years using Fisher exact tests and Mann-Whitney U tests, respectively.

### *Correlation between Lythrum and Sidalcea*

In Ladner marsh, eight areas were selected subjectively to include *Sidalcea*. A 20-m line was run subjectively through the vegetation, and the densities of *Sidalcea* and *Lythrum* were measured by counting the number of individual shoots of each species

that touched the line (hits). Care was taken to keep the line stretched at 1 m above the ground to ensure equal sampling in all areas. More lines were run parallel to the first one at a distance of 2 m. This procedure was repeated until the selected area was covered.

A total of 124 samples were taken. Four samples with zero scores for both species were not considered in this analysis. Pearson correlation analysis was used to test for an association between the two species. Counts were square root transformed prior to the statistical analysis to meet the assumptions of linear correlation.

A second analysis was done using the frequency data recorded in quadrats in 1979 and 1999, respectively (see Methods above) to determine whether the relationship between the two species changed over time. The data for 1979 and 1999 were analyzed separately using the Fisher exact test.

#### *Lythrum removal experiment*

To test the response of *Sidalcea* to a reduction in density of surrounding plants, an experiment was carried out from July 2000 to August 2002. Experimental plots were 1m<sup>2</sup> in size and contained both species, *Lythrum* and *Sidalcea*. To maximize treatment effects, I deliberately chose plots that contained a relatively high abundance of *Lythrum*. Three treatments were applied; 1) In the control treatment, no manipulation was carried out; 2) In the *Lythrum*-removal treatment, all *Lythrum* plants were killed; 3) In the native-removal treatment, native plants were killed, but not *Lythrum*, to compare the responses to removal of *Lythrum* to the removal of native species.

Nine replicates were used in each treatment, totaling 27 experimental plots. Plots were established in eight different areas of the marsh, labeled a - h. The areas

contained three plots each with the exception of one area, c, which contained six plots. Treatments were applied randomly to plots within the areas.

To remove plants, all leaves of shoots selected for removal were wiped with a systemic glyphosate herbicide, Round-up<sup>®</sup>. The solution was prepared according to the product label, and the efficacy of the treatment on *Lythrum* was confirmed by checking the removal plots for green *Lythrum* shoots two weeks after the treatment. Native plant removal plots were not checked since plants targeted for removal could not be distinguished from other individuals of the same species.

In the native removal treatment, one shoot of a randomly chosen native plant was wiped for every stem of *Lythrum* present in the plot. To imitate the spatial pattern of *Lythrum* in the plots, shoots next to a *Lythrum* stem were selected. The shoots were chosen subjectively and included different species of native plants, both annuals and perennials.

In July of 2000, prior to experimental treatments, *Lythrum* and *Sidalcea* total shoot lengths were recorded in each plot. In August of 2001 (year 1) and June of 2002 (year 2), the following variables of *Sidalcea* performance were recorded: total shoot length per plot; total number of leaves per experimental plot; inflorescence length of the five tallest shoots; inflorescence flower density (per 5 cm of inflorescence) of the five tallest shoots; and total number of inflorescences per plot. In 2002 only, *Lythrum* and *Sidalcea* above-ground biomass was harvested in each plot and dried to constant weight at 70 °C. Only above-ground variables were measured, since it was not possible to determine the below-ground performance of the plants without major disturbances (digging in the soil) to the plots.

## Statistical analysis

The vegetative and reproductive performance of the target plant was analyzed separately in each year, since the error structure varied between years. The data were analyzed using a regression model with treatment (control, *Lythrum* removal, native removal), areas (a – h) and *Sidalcea* and *Lythrum* total shoot lengths at the onset of the experiment (both log transformed) as factors. Dry biomass of the shoots and of the inflorescences in the experimental plots were considered as response variables according to the following model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \tau(X_{ij} - \bar{X}) + \varpi(Z_{ij} - \bar{Z}) + \varepsilon_{ij},$$

where  $Y_{ij}$  indicates the response of *Sidalcea* in each plot,  $\mu$  is the overall average response for the whole experimental population,  $\alpha_i$  is the effect of the  $i$ th treatment,  $\beta_j$  is the effect of the  $j$ th area,  $X_{ij}$  is the total *Sidalcea* shoot length at the onset of the experiment in each plot,  $\tau$  is the slope of the regression between Y and X,  $Z_{ij}$  is the total *Lythrum* shoot length at the onset of the experiment in each plot,  $\varpi_i$  is the slope of the regression between Y and Z, and  $\varepsilon_{ij}$  is a random error in the experiment.

Since dry biomass was only measured in 2002, *Lythrum* total shoot length and *Sidalcea* total shoot length (both log-transformed) were used as surrogates for dry biomass in all statistical analyses. Both variables were significantly correlated with dry biomass (*Lythrum*:  $r = 0.96$ ,  $n = 21$ ,  $P < 0.001$ , *Sidalcea*:  $r = 0.92$ ,  $n = 26$ ,  $P < 0.001$ ). Reproductive performance of *Sidalcea* was measured by the index  $R_i = n_i \cdot l_i$ , where  $n_i$  = number of inflorescence in the  $i$ th experimental plot, and  $l_i$  = average length of the inflorescences of the five tallest *Sidalcea* shoots in the  $i$ th experimental plot.  $R_i$  was

closely correlated with *Sidalcea* inflorescence dry biomass after one outlier was removed from the data ( $r = 0.92$ ,  $n = 25$ ,  $P < 0.001$ ).

Data were excluded from the analysis in the following cases. The herbicide application caused plant damage to *Sidalcea* in one plot (*Lythrum*-removal) in area h. In one additional plot (control), both *Lythrum* and *Sidalcea* were strongly reduced in year 1, possibly due to disturbances in the winter time since the plot was at the edge of a drainage channel. To balance the data structure, all plots in area h were excluded from the analysis, and the measurements of the plots in area c were averaged after excluding the outlier. In total, data from 21 plots in seven areas were analyzed.

#### *Pot experiment*

I also carried out a pot experiment on a greenhouse patio to compare field and laboratory results of the relative competitive effect of *Lythrum* on *Sidalcea*. The design was additive, with one *Sidalcea* individual as target plant, grown together with or without (control) competitor plant species. The competitive effect of the competitors, i.e. their ability to suppress the target plant, was evaluated by comparing the performance of the target plants grown with the different competitors (Goldberg and Scheiner 1993, Keddy et al. 2000, Weigelt and Jolliffe 2003). Apart from *Lythrum*, two perennial native plant species were also selected as competitors, a willow, *Salix lasiandra* Beth., and Pacific water parsley, *Oenanthe sarmentosa* Presl. Both are commonly found growing together with *Sidalcea* in the marsh and can easily be propagated. The ramets were harvested in early May and taken to the greenhouse at the University of British Columbia. *Salix* cuttings, approximately 10 cm in length, and *Oenanthe* seedlings were grown in plant pots containing Perlite<sup>®</sup> (Westgro Sales Inc., Delta) until the end of May, when all had

well-established roots. The *Sidalcea* plants were grown from seeds collected in the fall of 1999 from a population close to Duncan, British Columbia (Vancouver Island), because seeds were more abundant in this population. At the end of March 2000, the seeds were scarified with sandpaper, treated with a fungicide (No-Damp<sup>®</sup>, Plant Products Co. Ltd, Brampton) and stratified at 4°C (i.e., the seeds were placed in a small container with moist sand and were left in a refrigerator) for two weeks to break dormancy (Marshall 1998). Seeds were germinated in flats filled with mineral soil. All ramets were grown in a mist bed in the University of British Columbia's greenhouse, where water sprinklers and heating cables for bottom heat maintain a humid (80 % relative humidity) and warm (25°C) climate to encourage plant growth.

To establish the experiment, I filled 4 L (150 mm W x 180 mm L x 150 mm H) plastic plant pots with drainage holes with mineral soil. Individual *Sidalcea* target plants, ranging in size from the two- to five-leaf stage, were arbitrarily selected, placed in the center of the pot and surrounded by a circle of a variable number (zero to eleven) of one competitor plant species to create a range of competitor densities (Goldberg and Scheiner 1993). The competitors were placed halfway between the target plant and the edge of the plant pot.

In a factorial design, competitor densities chosen were zero (control), two, four, six, eight, or eleven individuals. The natural density of *Lythrum* in the marsh area where it co-occurs with *Sidalcea* corresponds to 0.16 stems per plant pot, given the pot surface. The relationship between competitor density and target plant performance is expected to be hyperbolic or inverse linear, indicated by a sharp drop in target plant performance at low competitor densities and a slow decline at high competitor densities (Weigelt et al. 2002).

Five replicate pots per treatment combination of species and density were used totaling 30 pots per competitor, except for *Oenanthe* where two additional replicates were set up, resulting in a total of 32 pots. On June 1, 2000, the pots were placed in an open wooden frame outdoors on the greenhouse patio. The positions of the treatments were randomized. Each pot was encircled by chicken wire up to 50 cm in height to discourage the plants from growing outside their pots.

All pots were watered daily using a 120 ppm nutrient solution of Peters<sup>®</sup> Excel<sup>®</sup> 15-5-15 Cal Mag<sup>®</sup> (Jack van Klaveren, Delta, British Columbia), which contains a complete package of NPK, MgO and trace elements. This concentration is sufficient for the growth requirements of most plants during the summer months.

The experiment was carried out over one full growing season and into the early summer of the second growing season until flowering of the target plant started (mid-June). In August of 2001, year 1, plant performance was estimated by recording the total number of leaves. The plants were then left on the greenhouse patio over the winter. In January of 2002, I harvested the aboveground biomass of all plants and dried it to constant weight at 70°C.

In June of 2002, year 2, target plant performance (regrowth) was estimated again using the same parameters as before; above-ground biomass of all surviving plants was harvested and dried to constant weight as before.

### Statistical analysis

Responses in year 1 and year 2 were analyzed separately because overwintering mortality caused a loss of 18 replicates and would have reduced statistical power. With the data in year 1, I carried out a two-factor analysis of variance

with competitor and density as factors. This reflects the design of the experiment and effectively removes between-subject variability without the assumption of a linear relationship between the quantitative blocking factor and the response variable (Huitema 1980). Competitor density was at least marginally related to dry biomass in all competitor species in 2001 (*Lythrum*,  $r_s = 0.39$ ,  $n = 24$ ,  $P = 0.06$ ; *Oenanthe*:  $r_s = 0.59$ ,  $n = 22$ ,  $P < 0.01$ ; *Salix*:  $r_s = 0.41$ ,  $n = 24$ ,  $P = 0.04$ ), and thus, density was considered an appropriate measure of plant abundance.

The experimental design was not a complete factorial, because the zero level (control) of the density factor did not differ between competitors, and thus, the performance of the target plant should not vary when it is grown without competitors. Therefore, I did not consider the control treatment replicates in the statistical analysis of treatment effects, but only used them to confirm the efficacy of the density treatments.

I used  $\sqrt{(\text{total number of leaves})}$  as a measure of plant performance in both years, because the number of leaves was recorded consistently in each year.  $\sqrt{(\text{total number of leaves in 2002})}$  was strongly correlated with  $\log(\text{plant dry biomass in 2002} + 0.5)$  ( $r = 0.95$ ,  $n = 67$ ,  $P < 0.001$ ), a measure of plant performance commonly used in plant competition studies (e.g. Keddy et al. 2000).

With the data in year 2, I carried out two separate, non-parametric one-factor analyses (Kruskal-Wallis), assuming no interaction between competitor and density, because transformations did not improve the heteroscedasticity, and the data structure was unbalanced. The analysis was repeated with competitor biomass as the second factor, since there was no relationship between density and biomass suggesting that competitor mortality had reduced the initial competitor density, and that competitor density was not an accurate measure of competitor plant abundance. Six replicates, two

from each competitor species, were set aside for another experiment and not used in year 2.

### *Segregation of Lythrum and Sidalcea*

I documented the spatial and temporal segregation of *Lythrum* and *Sidalcea* in the marsh and carried out a series of experiments to investigate the roles of environmental conditions and seed limitation as causes for the spatial segregation. The sampling and the experiments were carried out at an easily accessible site of the marsh, approximately 50 m South of the viewing tower at Ladner marsh. In the area in the West, the soil is constantly waterlogged, but in the area in the East, the soil is well-drained at low tide. In the well-drained, but not in the waterlogged area, *Sidalcea* occurs, and *Lythrum* density is higher in the waterlogged area than in the well-drained area, when compared visually.

### Spatial segregation

To document the spatial distribution of *Lythrum* and *Sidalcea*, I recorded the densities of both species in the waterlogged and in the well-drained area using line-sampling (as in the correlation study). In the waterlogged area, *Lythrum* density was also estimated using quadrat sampling (as in the survey).

## Temporal segregation

To determine differences in emergence time between the two species, 20 *Lythrum* and *Sidalcea* shoots were measured four times from May to August of 2001 in the well-drained area. The area appeared to be homogeneous with respect to the vegetation. To ensure random sampling, the shoots were selected as follows: from an arbitrary starting point I walked 5 m along a straight line in an arbitrary direction subject to the condition that I remained within the sampling area. At this position, I selected the closest individuals of both plant species and measured the height of their tallest shoots. The sampling process was continued in the manner mentioned above until 20 shoots were sampled. Mean shoot heights were compared using Mann-Whitney U tests for the samples at each datum.

## *Transplant experiment*

On May 24, 2001, 30 individual *Lythrum* plants were transplanted into Ladner marsh. The plants had been grown from stem cuttings in the University of British Columbia's greenhouse and all had a single shoot. In an incomplete factorial design, the plants were randomly assigned to three different treatments, defined by transplant area (waterlogged vs. well-drained) and removal (removal treatment in the well-drained area only vs. no-removal treatment in both areas).

Ten plants were transplanted to the waterlogged area. The remaining 20 plants were transplanted into the well-drained area. Half of the plants in the well-drained area were transplanted individually into the center of 0.25 m<sup>2</sup> openings in the vegetation, created by removing all above-ground vegetation and severing the roots in the area with

a spade (removal treatment). The remaining ten plants were transplanted individually into the natural vegetation without prior manipulation (no-removal treatment). The plants were systematically arranged at intervals of 2 m along a straight line to facilitate locating them in the future. To differentiate the transplanted stems from naturally growing stems, the experimental plants were marked with a dot of Liquid Paper® on one leaf.

Three times, on June 14, July 18 and August 14, 2001, the height and survival of the transplants were assessed. Effects of area (waterlogged vs. well-drained) and vegetation removal (removal vs. no-removal) on plant height were analyzed using repeated measures analysis of variance with two among-subjects factors, area and removal treatment, and one within-subjects factor, time, with three levels (Zar 1999). Since the experimental design was incomplete factorial, i.e. the removal treatment was not carried out in the waterlogged area, I had to assume no interaction between area and removal treatment. All shoot measurements were log-transformed.

#### *Lythrum seed availability*

To examine whether the varying density of *Lythrum* in the waterlogged and well-drained area, respectively, is due to limited seed availability, I analyzed the dependence of seedling emergence on the area (waterlogged vs. well-drained) and removal treatments (removal vs. no-removal) outlined in the experiment above. In August of 2001 and in June of 2002, the emergence of seedlings in 0.25 m<sup>2</sup> experimental plots around the transplants was recorded. I used contingency testing after pooling the treatments in which no openings were created, when they did not differ significantly.

### 3. Results

#### *Changes in species densities from 1979 to 1999*

In 1979, *Lythrum* was recorded in 31 of 100 quadrats. Although not statistically significant, the frequency increased by 19 % to 37 quadrats in 1999 (Fisher exact test, right tail:  $P = 0.23$ ) as did the ground cover from  $7.9 \pm 1.6$  % (mean  $\pm$  SEM) to  $8.6 \pm 1.5$  % ( $Z = 0.88$ ,  $P = 0.19$ ). The frequency of *Sidalcea* decreased from 15 to seven quadrats (Fisher exact test, left tail:  $P = 0.06$ ), and similarly, the ground cover of the species decreased from  $2.7 \pm 0.9$  % to  $2.1 \pm 0.9$  % ( $Z = -1.70$ ,  $P = 0.04$ ).

#### *Correlation between Lythrum and Sidalcea*

The density of *Sidalcea* in the marsh was  $2.5 \pm 0.31$  (mean  $\pm$  SEM) counts per line, and the average *Lythrum* density in areas with *Sidalcea* was  $5.5 \pm 0.38$  counts per line. Using the line-data, *Lythrum* and *Sidalcea* densities were negatively correlated ( $r = -0.22$ ,  $n = 120$ ,  $P = 0.02$ ); however, considerable variation in the data (Fig. 2-1) resulted in only a low correlation coefficient.

The line-sampling result differed from that obtained using the 1 m<sup>2</sup> quadrat data, where no negative association between *Lythrum* and *Sidalcea* was detected, either in 1979 (Fisher exact test, left tail:  $P = 0.48$ ) or in 1999 (Fisher exact test, left tail:  $P = 0.19$ ; Table 2-1).

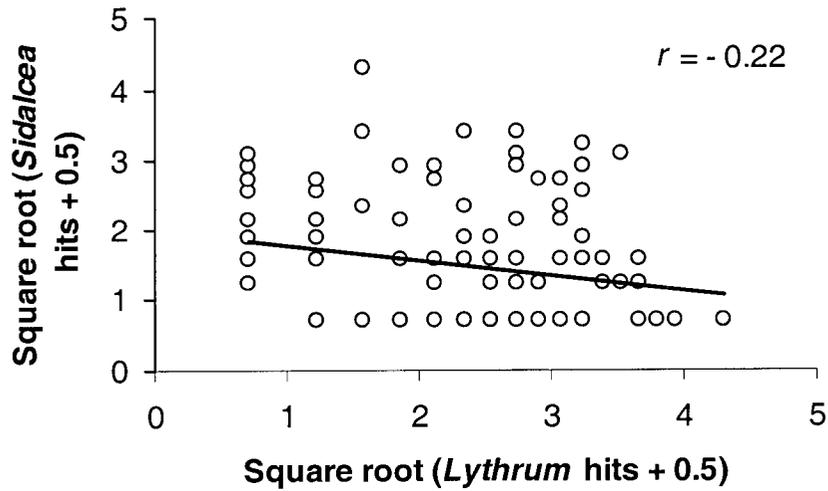
## *Lythrum* removal experiment

On average, *Lythrum* abundance before the onset of the experiment was  $1913 \pm 238$  cm of shoot length per plot. A 95 % reduction of *Lythrum* in the *Lythrum*-removal treatment in year one and a 98.5 % in year two indicated that *Lythrum* was effectively removed from the treated plots. The average shoot length of *Sidalcea* was  $1428 \pm 254$  cm, and was higher in the control than in the *Lythrum* and native plant removal plots ( $F_{2, 18} = 4.33$ ,  $P = 0.03$ ).

In year 1, the highly significant regression revealed that the model (Table 2-2) could accurately predict *Sidalcea* abundance ( $F_{10, 20} = 11.18$ ,  $P < 0.001$ ). The adjusted *Sidalcea* abundance was marginally higher in the *Lythrum* removal treatment when compared to the control and the native plant removal treatment (Fig. 2-2A). Only *Sidalcea* abundance before the onset of the experiment, but not that of *Lythrum*, had a significant effect on its own performance (Table 2-2).

The overall regression model in year 2 was not significant ( $F_{10, 20} = 1.47$ ,  $P = 0.28$ ), indicating that the model could not adequately predict *Sidalcea* abundance (Table 2-3). *Sidalcea* abundance was only affected by its own abundance at the beginning of the experiment, but not by treatment, area or *Lythrum* abundance. The variability in the response was higher in year 2 than in year 1 (Fig. 2-2A).

When considering the reproductive output of *Sidalcea*, measured by  $R_i$ , the overall regression model was not significant in either year (year 1:  $F_{10, 20} = 2.09$ ,  $P = 0.13$ ; Table 2-4; year 2:  $F_{10, 20} = 0.95$ ,  $P = 0.53$ ; Table 2-5). Only the abundance of *Sidalcea* at the beginning of the experiment was significant in year 1 (Table 2-4, Fig. 2-2B), while none of the predictor variables were significant in year 2 (Table 2-5, Fig. 2-2B).



**Fig. 2-1** Scatterplot between *Lythrum* and *Sidalcea* densities (hits) in Ladner marsh. Hits are the number of times individual shoots touch a 20 m-sampling line (see Methods). The relationship is significant ( $P = 0.02$ ).

**Table 2-1** Frequency of *Lythrum* and *Sidalcea* occurrence in 100 1m<sup>2</sup> plots

(quadrats) in 1979 and 1999. There was no association between the two species either in 1979 (Fisher exact test, left tail:  $P = 0.48$ ) or in 1999 (Fisher exact test, left tail:  $P = 0.19$ ).

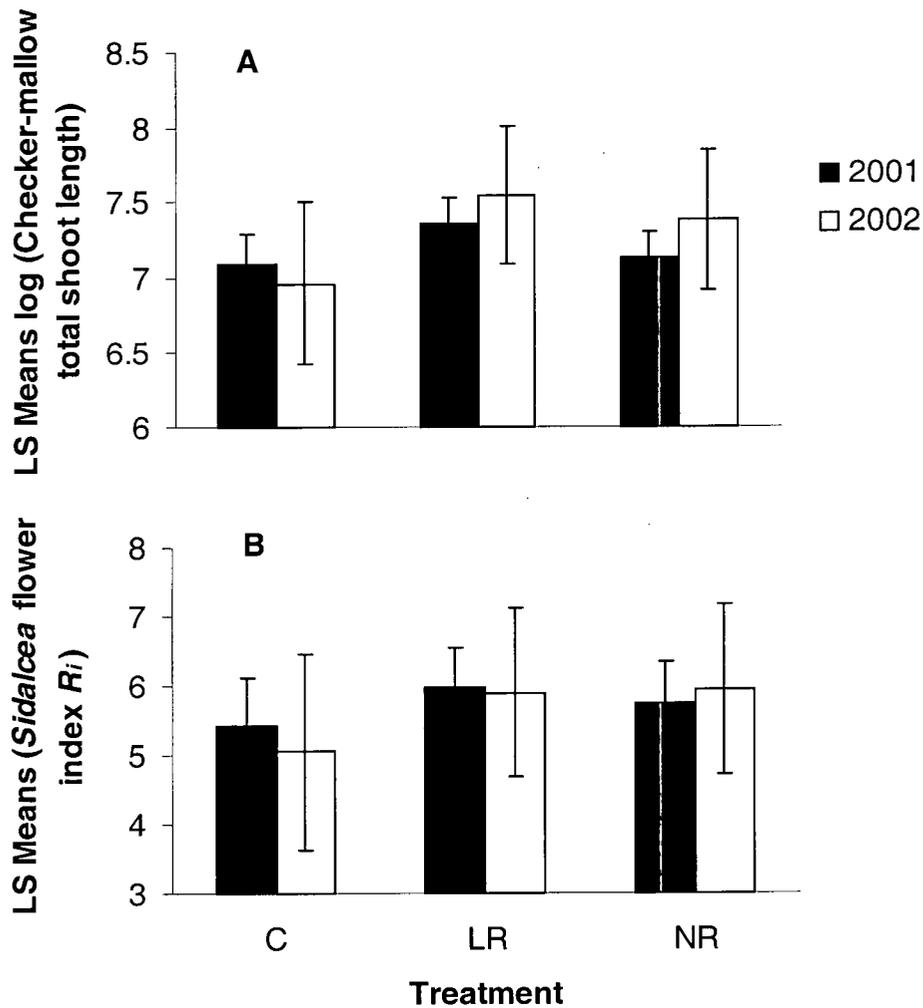
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Presence of Species		Year	
<i>Lythrum</i>	<i>Sidalcea</i>	1979	1999
Yes	Yes	4	1
No	Yes	11	6
Yes	No	27	36
No	No	58	57

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**Table 2-2** Regression testing the effects of treatment (Control, *Lythrum* rem. = *Lythrum* removal, Native rem. = Native plant removal), area, and of the total initial shoot lengths of *Sidalcea* and *Lythrum* on the total shoot length of *Sidalcea* in year 1. All continuous variables were log transformed.

Source of variation	<i>df</i>	<i>SS</i>	<i>F</i>	<i>P</i>
Removal treatment	2	0.236	3.212	0.084
Contrast Control vs. <i>Lythrum</i> rem.	1	0.155	4.214	0.067
Contrast <i>Lythrum</i> rem. vs. Native rem.	1	0.172	4.672	0.056
Area	6	0.305	1.386	0.309
Total initial <i>Lythrum</i> shoot length	1	0.004	0.050	0.828
Total initial <i>Sidalcea</i> shoot length	1	1.621	44.143	0.000
Error	10	0.367		
Total	20	4.474		



**Fig. 2-2** *Sidalcea* (A) vegetative performance ( $\pm$  95 % CI) and (B) reproductive performance ( $\pm$  95 % CI) by treatment in year 1 and year 2 of the experiment. Treatment defined as follows: C – control, LR – *Lythrum* removal, NR – Native Plant removal; adjusted means of *Sidalcea* vegetative performance between the *Lythrum* removal treatment and the other treatments differed marginally in year 1, but not in year 2; *Sidalcea* reproductive performance did not differ between treatments in either year.

**Table 2-3** Regression testing the effects of plant removal treatment (Control, *Lythrum* removal, and Native plant removal), area, and of the total initial shoot lengths of *Sidalcea* and *Lythrum* on the total shoot length of *Sidalcea* in year 2. All continuous variables were log transformed.

Source of variation	<i>df</i>	<i>SS</i>	<i>F</i>	<i>P</i>
Treatment	2	0.733	1.422	0.286
Area	6	1.111	0.718	0.645
Total initial <i>Lythrum</i> shoot length	1	0.004	0.017	0.898
Total initial <i>Sidalcea</i> shoot length	1	1.933	7.500	0.021
Error	10	2.578		
Total	20	6.353		

**Table 2-4** Regression testing the effects of treatment (Control, *Lythrum* removal, and Native plant removal), area, and of the total initial shoot lengths of *Sidalcea* and *Lythrum* on the reproductive performance of *Sidalcea*, measured by  $R_i$  (see Methods), in year 1. Shoot lengths were log transformed, and the response variable,  $R_i$ , was square root transformed.

Source of variation	<i>df</i>	<i>SS</i>	<i>F</i>	<i>P</i>
Treatment	2	0.62	0.74	0.50
Area	6	5.18	2.06	0.15
Total initial <i>Lythrum</i> shoot length	1	0.84	1.99	0.19
Total initial <i>Sidalcea</i> shoot length	1	3.55	8.47	0.02
Error	10	4.20		
Total	20	12.99		

**Table 2-5** Regression testing the effects of treatment (Control, *Lythrum* removal, and Native plant removal), area, and of the total initial shoot lengths of *Sidalcea* and *Lythrum* on the reproductive performance of *Sidalcea*, measured by  $R_i$  (see Methods), in year 2. Shoot lengths were log transformed, and the response variable,  $R_i$ , was square root transformed.

Source of variation	<i>df</i>	<i>SS</i>	<i>F</i>	<i>P</i>
Treatment	2	1.90	0.53	0.60
Area	6	14.99	1.40	0.30
Total initial <i>Lythrum</i> shoot length	1	1.29	0.73	0.41
Total initial <i>Sidalcea</i> shoot length	1	3.91	2.20	0.17
Error	10	17.82		
Total	20	34.67		

## *Pot experiment*

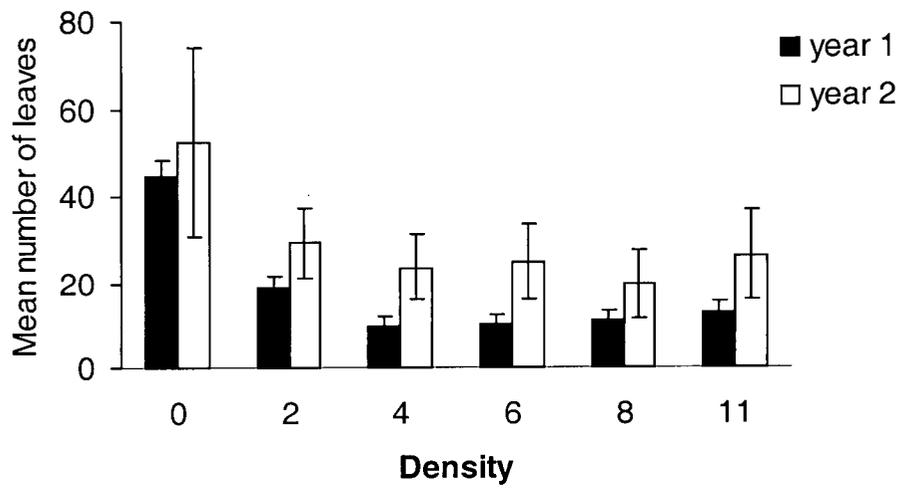
### Effect of competitor density on *Sidalcea* performance

Before examining the effect of competitor identity, it was necessary to confirm that adding competitor plants had an effect on *Sidalcea* performance. *Sidalcea* individuals produced, on average, 72 % and 53 % more leaves, in year 1 and year 2, respectively, in the control treatment than in all other species treatments combined (Fig. 2-3).

#### Year 1

In year one, five target plants died (one in the *Lythrum* treatment, four in the *Oenanche* treatment and one in the *Salix* treatment) and were not considered in the analysis. One data point (*Salix* treatment) was considered an outlier because its residual was much greater than that of the remaining data points, and removed from the data. A total of 70 target plants were included in the analysis, 24 in the *Lythrum* treatment, 23 in the *Oenanche* treatment, 23 in the *Salix* treatment. The average leaf production per *Sidalcea* plant, excluding the controls, was  $12.6 \pm 1.4$  leaves per plant.

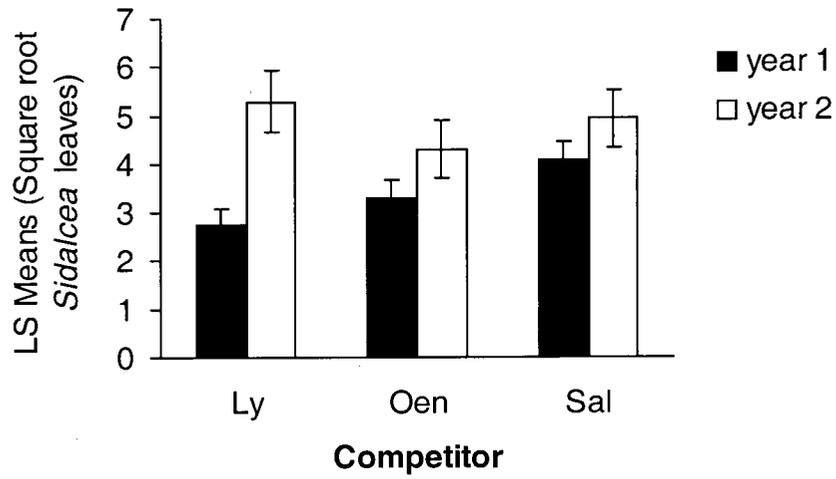
Both competitor species and density had significant effects on *Sidalcea* performance (Table 2-6). There was no significant interaction between competitor species and density. Considering only the target plants that survived, the effect of *Lythrum* on the target plant performance was stronger than the effect of *Oenanche* (Contrast Ly vs. Oen) or the effect of *Salix* (Contrast Ly vs. Sal) (Fig. 2-4), as indicated by the greatest reduction in *Sidalcea* leaf production.



**Fig. 2-3** *Sidalcea* performance (number of leaves  $\pm$  95 % CI), averaged over all competitor species, by competitor density in both years of the pot experiment.

**Table 2-6** Two-factor Analysis of Variance testing the effect of competitor (Ly = *Lythrum*, Oen = *Oenanthe*, Sal = *Salix*) and density on the  $\sqrt{}$  (total number of *Sidalcea* leaves) in year 1 of the pot experiment.

Source of variation	<i>df</i>	<i>SS</i>	<i>F</i>	<i>P</i>
Density	4	15.95	5.82	< 0.001
Competitor	2	20.85	15.22	< 0.001
Contrast Ly vs. Oen	1	3.45	5.04	0.029
Contrast Ly vs. Sal	1	20.70	30.22	<0.001
Competitor x Density	8	6.43	1.17	0.331
Error	55	37.68		
Total	69	80.91		



**Fig. 2-4** Least Squares Means ( $\pm$  95 % CI) of the  $\sqrt{\text{Number of } Sidalcea \text{ leaves}}$ , adjusted for density effects, by competitor species in year 1 and year 2 of the pot experiment; Treatments defined as follows: Ly - *Lythrum*, Oen - *Oenanthe*, Sal - *Salix*.

Differences in competitive effects on *Sidalcea* were not simply due to differences in above-ground biomass among competitors, since *Salix* was the competitor species with the least suppressive effect despite its high biomass. When weighted by the number of replicates in each density treatment, the average dry biomass of *Salix* was  $38.8 \text{ g} \pm 2.25 \text{ g}$ , followed by *Lythrum* with  $28.0 \text{ g} \pm 1.20 \text{ g}$  and *Oenanthe* with  $23.9 \text{ g} \pm 2.77 \text{ g}$ .

## Year 2

Due to the warm conditions on the greenhouse patio, most of the experimental plants emerged early. In early February of 2002, 86 % of all *Sidalcea* plants had emerged. Similarly, in 83 % of the pots with *Lythrum*, and in all of the pots with *Oenanthe*, the competitors had grown leaves by this time, although none of the *Salix* plants had emerged.

Overall target plant mortality was similar when in competition with *Lythrum* and *Oenanthe*, but lower for *Salix*, with eight, seven and three target plants, respectively, dying over the course of the experiment. In three cases, the competitor species died. These target plants were discarded and not used in the analysis. In addition, one data point was considered an outlier and was removed from the data, because its residual variation was much greater than that of the remaining data.

A total of 50 replicates were analyzed, 15 in the *Lythrum* treatment, 17 in the *Oenanthe* treatment and 18 in the *Salix* treatment. The average number of leaves per target plant almost doubled in year 2 to  $24.1 \pm 2.2$  leaves per plant (Fig. 2-3), indicating increased vigor of the surviving plants (paired-t test,  $t_{14} = 5.14$ ,  $P < 0.001$ ).

Competitor species (Kruskal-Wallis,  $\chi^2_2 = 6.00$ ,  $P = 0.05$ ), but not density, influenced *Sidalcea* performance ( $F_{4, 46} = 0.79$ ,  $P = 0.54$ ). Similarly, when dry biomass was used rather than density in the analysis of variance, target plant performance was only dependent on the competitor species (Table 2-7). The mean performance of the target plant was highest when grown with *Lythrum*, but lower when grown with the native competitors (Fig. 2-4). Hence, the competitive effect of *Lythrum* was less than the effect of either native plant species, despite the observation that the average dry biomass of *Lythrum* in the experimental containers greatly exceeded that of either native species.

The average dry biomass of *Lythrum* in year 2 ( $28.8 \pm 3.8$  g) was comparable to the dry biomass of *Lythrum* in year 1 (paired-t test,  $t_{14} = 0.71$ ,  $P = 0.49$ ); however, the average dry biomass of both native plants was considerably smaller in year 2 than in year 1 with  $9.7 \pm 1.8$  g in *Oenanth*e (paired-t test,  $t_{15} = -5.03$ ,  $P < 0.001$ ) and  $9.5 \pm 1.7$  g in *Salix* (paired-t test,  $t_{18} = -8.99$ ,  $P < 0.001$ ), corresponding to an average reduction in dry biomass of 59 % and 69 %, respectively. In *Lythrum* and *Oenanth*e, there was no correlation between density and dry biomass (*Lythrum*:  $r_s = -0.28$ ,  $n = 15$ ,  $P = 0.30$ ; *Oenanth*e:  $r_s = 0.32$ ,  $n = 18$ ,  $P = 0.20$ ), but in *Salix*, a strong, negative relationship with density was found ( $r_s = -0.59$ ,  $n = 18$ ,  $P < 0.01$ ).

**Table 2-7** Regression testing the effect of competitor (Ly = *Lythrum*, Oen = *Oenanthe*, Sal = *Salix*) and dry biomass (g) (untransformed) on the total number of leaves in *Sidalcea* individuals (untransformed) in year 2 of the pot experiment.

Source of variation	<i>df</i>	<i>SS</i>	<i>F</i>	<i>P</i>
Competitor	2	794.93	3.27	0.048
Contrast Ly vs. Oen	1	788.88	6.49	0.015
Contrast Ly vs. Sal	1	274.45	2.26	0.14
Dry biomass	1	269.93	2.22	0.144
Competitor x Dry biomass	2	197.28	0.81	0.451
Residual	43	5229.52		
Total	48	6314.12		

## *Segregation of Lythrum and Sidalcea*

### Spatial segregation

In the waterlogged area, *Lythrum* density averaged  $20.8 \pm 1.7$  (mean  $\pm$  SEM) counts  $\cdot$  line<sup>-1</sup>. Using square quadrats I estimated the density of *Lythrum* here to be  $19.3 \pm 2.6$  stems per m<sup>2</sup>.

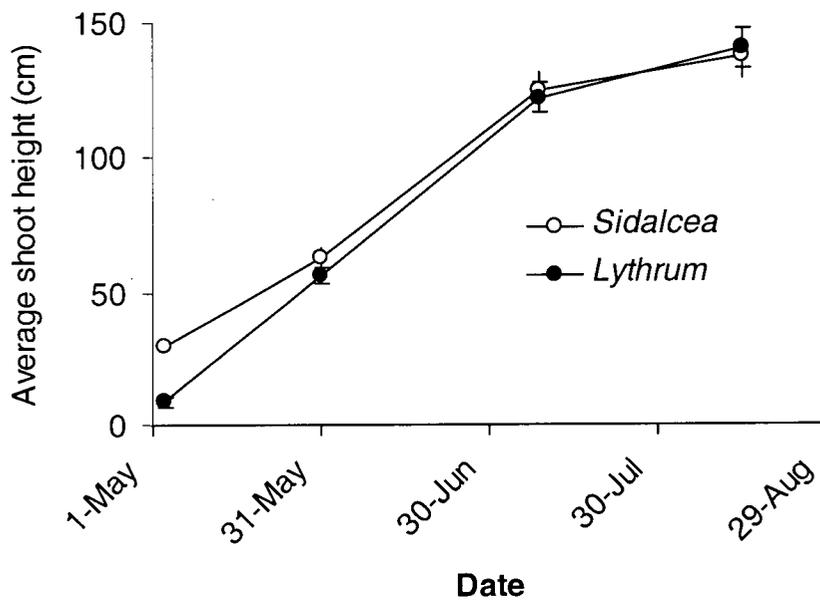
In the well-drained area, *Lythrum* average density was only  $9.7 \pm 1.0$  counts  $\cdot$  line<sup>-1</sup>. Thus, in the area with *Sidalcea*, *Lythrum* is only half as abundant as in the waterlogged area.

### Temporal segregation

The shoots of adult *Sidalcea* individuals were consistently taller than *Lythrum* shoots (Fig. 2-5) until June of 2001 (May 3:  $\chi^2 = 29.35$ ,  $P < 0.001$ ; May 31:  $\chi^2 = 7.41$ ,  $P < 0.01$ ). In the two later measurements, the shoot heights of the two species did not differ significantly (July 9:  $\chi^2 = 0.36$ ,  $P = 0.55$ ; August 14:  $\chi^2 = 0.003$ ,  $P = 0.96$ ).

### *Transplant experiment*

Seven (23 %) of the *Lythrum* plants disappeared from the transplant sites, two from the waterlogged site without removal treatment, three from the well-drained location without removal treatment, and two from the well-drained location with removal treatment. Averaged over the three sampling dates, *Lythrum* shoots in the removal

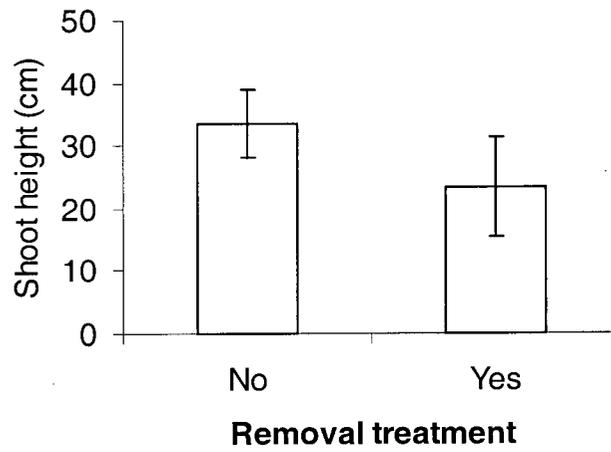


**Fig. 2-5** Average height ( $\pm 95\%$  CI) of randomly selected shoots of *Sidalcea* and *Lythrum* in Ladner Marsh on four sampling dates (May 3, May 31, July 9, August 14) in 2001; *Sidalcea* shoots were significantly taller than *Lythrum* shoots in early and late May, but not thereafter (see Results).

treatment were 31 % smaller than shoots in non-removal plots (Fig. 2-6). Average *Lythrum* shoot height differed significantly between removal treatments, but not between areas (Table 2-8). The average increase of 15 cm from June to August indicates a significant growth of *Lythrum* shoots over time, and shoot growth differed between the two areas as indicated by the significant time by area interaction.

#### *Lythrum seed availability*

Openings in the vegetation encouraged *Lythrum* seedling germination. In 2001 and 2002, five and three of the ten artificial openings, respectively, had flushes of *Lythrum* seedlings (10+ individuals) appear, but there were no *Lythrum* seedling flushes in locations where no vegetation was removed (Fisher exact test, 2001:  $P = 0.02$ ; 2002:  $P = 0.11$ ).



**Fig. 2-6** Average height ( $\pm$  95 % CI) of *Lythrum* shoots transplanted into areas with (n = 8) and without removal (n = 15) of the surrounding vegetation; shoots were taller when the surrounding vegetation was not removed ( $F_{1,20} = 5.00$ ,  $P = 0.04$ ).

**Table 2-8** Repeated Measures Analysis of Variance testing the effects of plant removal (removal vs. no removal) and area (waterlogged vs. well-drained area) on the shoot height of *Lythrum* individuals (log transformed) on June 14, July 18 and August 14, 2001. No interaction between the between-subject factors was assumed (see Methods).

Source of variation	<i>df</i>	<i>SS</i>	<i>F</i>	<i>P</i>
Between-Subjects	22			
Area (A)	1	0.10	0.32	0.58
Removal (R)	1	1.61	5.00	0.04
Plots <small>within AR</small>	20	6.40		
Within-Subjects	46			
Time (T)	2	5.10	63.37	0.00
T x A	2	0.32	3.99	0.03
T x R	2	0.04	0.51	0.61
T x Plots <small>within AR</small>	40	1.60		
Total	68			

## **4. Discussion**

### *Changes in species densities from 1979 to 1999*

The invasion of *Lythrum* into Ladner marsh seems to have been largely completed by 1979 and an increase in abundance may not occur under the current environmental conditions, since *Lythrum* abundance did not increase significantly from 1979 to 1999. Thus, the decrease in *Sidalcea* abundance in both frequency and ground cover suggests that the decrease of the native plant is not due to the invader plant, although I cannot discount that an increase in the frequency of the invader plant by 19 % could affect *Sidalcea* if strong competition between the two species occurs. However, evidence from my studies (below) does not support the hypothesis that *Lythrum* significantly affects the native *Sidalcea* in Ladner marsh.

### *Correlation between Lythrum and Sidalcea*

The results of the observational studies concerning the distribution of the two species varied with the method used. While the data collected using 1m<sup>2</sup> quadrats did not provide evidence for a relationship between the two species, the line-data showed a negative association indicating that the two species are spatially segregated. This pattern could be explained by either of two competing hypotheses, the competition hypothesis and the habitat-preference hypothesis.

The negative relationship could indicate that the two species exclude each other from the same microhabitat due to competition for resources. Both species are tall growing and perennial, and may compete for root space, access to light (Keddy 2000), nutrients (Bedford et al. 1999) and pollinators (Grabas and Lavery 1999, Brown and Mitchell 2001).

Alternatively, the negative relationship could also be a consequence of different habitat preferences of the two species. Although I selected areas in which both species occurred, I may have sampled different community types (Bradfield and Porter 1982) within the marsh, which may not be equally optimal for the growth of both species. Plant communities in Ladner marsh do not show clear boundaries, but are intermixed, and subtle physical and chemical habitat differences that were not obvious to me could lead to a negative association, reflecting spatial segregation due to environmental differences. Although I did not find a significant effect of area on *Sidalcea* shoot height in the transplant experiment (see Results below), this experiment lasted only over a short period of time and was carried out in only two different habitat types (areas). Thus, the pattern found in the line-data could reflect subtle differences in habitat preferences between the two species.

The problem of confounding habitat preferences and competitive displacement is also present when sampling quadrats across the marsh. In fact, the problem may be even more severe, because I clearly sampled different habitats corresponding to different vegetation zones within the marsh. Replicating Bradfield and Porter's sampling design (1982), my survey included

habitats that differ primarily in their hydrological regime. Yet, despite the heterogeneous environments in which I sampled, there was no evidence for a significant relationship between the two species. However, only 15 and seven of the 100 quadrats sampled in 1979 and 1999, respectively, contained *Sidalcea*. Based on the low frequency of occurrence of *Sidalcea*, it may not be reasonable to draw conclusions regarding the distribution of the two species.

In summary, the line-sampling results address the question more appropriately because of the more homogeneous environment in which the sampling occurred. The negative relationship between the two species found in the line-data is necessary, but not sufficient to support the hypothesis that the two species are competing.

#### *Lythrum removal experiment*

Despite the fact that the experimental plots were deliberately chosen such that they contained a high density of *Lythrum* and that the two species occurred intermixed within the plots, the response of *Sidalcea* to the *Lythrum* removal treatment was weak and transient. *Lythrum* removal only marginally affected *Sidalcea* performance in year 1, and not at all in year 2. The abundance of *Lythrum*, measured by the total shoot length, did not have an effect on *Sidalcea* performance in either year. These results suggest that *Lythrum* is not an important factor in determining the performance of *Sidalcea*.

If competition is in fact weak or absent, it may be due to the fact that *Lythrum* did not reach so high an abundance as to affect *Sidalcea* performance, and thus, its removal did not result in a strong response. This is consistent with the result that *Lythrum* above-ground abundance, measured by the total shoot length and taken into account as an independent factor in the analysis, was not significant. The density of *Lythrum* in the well-drained areas in which *Sidalcea* occurs may be low because the environmental conditions (see Discussion below) do not allow the invader plant to reach higher density.

Alternatively, it has been pointed out that strong interspecific competition may lead to spatial segregation of two species, and consequently, competition would be difficult to document through simple removal experiments (Freckleton and Watkinson 2000a, b). In cases of spatial segregation between competitors a low response to the removal treatment does not necessarily indicate that competition between the two species is weak. However, if competition is in fact so strong that it exceeds competition between *Sidalcea* and native species, I expect this result to have been obvious in the pot experiment, where competitors were added to the target plant. Yet, this was not what I observed.

In contrast to the marginal effect of *Lythrum* removal, the removal of the native vegetation had no effect on *Sidalcea* in either year. The similar response in the native removal and control treatments could indicate that the gaps created by native plants are not sufficient to allow a significant increase in *Sidalcea* growth. Possibly, *Lythrum*'s tall shoot growth or its large rootstock in comparison with many native species account for the difference in response. Alternatively,

the native removal treatment was not as sustained as the *Lythrum* removal treatment because the removal included annual species that die at the end of the growing season and are replaced by seedlings in the following year. Their one-time removal at the end of the growing season is not likely to affect the remaining plant species, if their proportion among the plant species is high.

While plant removal treatments had almost no predictive power for the performance of *Sidalcea*, the initial abundance of *Sidalcea* itself explained most of the variation in target plant abundance in both years. This could be a reflection of the perennial life-style and clonal growth of *Sidalcea*: new shoots arise from existing plants, and apparently, the average annual *Sidalcea* mortality is not high. The observation that the initial abundance of *Sidalcea* differed between treatments despite the random assignment of the plots to the treatments does not affect the result because the statistical analysis adjusts the means of the dependent variable by an appropriate amount (Huitema 1980).

#### *Pot experiment*

Despite the limitations of greenhouse experiments, particularly their lack of realism, it has been pointed out that if competition cannot be shown under greenhouse conditions it is unlikely to be important in nature (Gibson et al. 1999). Since disturbances and stress are usually absent or insignificant under greenhouse conditions, plant biomass is generally higher than under natural conditions and thus, competition is expected to be more intense.

*Lythrum* had the most severe effect on *Sidalcea* seedlings (year 1 of the experiment) when compared with the impact of either of the two native species. This is consistent with the results of a similar study (Keddy et al. 2000), in which *Lythrum* was found to be the species which suppressed the target species the most under high nutrient conditions after one growing season. However, overall *Sidalcea* survival was similar among target plants grown with *Lythrum* or *Oenanthe*, and a possible, lower reproductive success of *Sidalcea* grown with *Lythrum* in the first year was likely small compared to the lifetime reproductive output of a perennial.

In year 2, the competitive effect of all competitor species was lower when compared with the effects in year 1, as shown by the better performance of the surviving *Sidalcea* plants in year 2. Although *Lythrum* produced the highest average dry biomass compared with the native competitor species, the competitive effect of *Lythrum* on *Sidalcea* was the least among the species tested.

Interestingly, the dry biomass in year 1 was much higher than in year 2 for both native species, but was comparable for *Lythrum*. In the case of *Salix*, this may have been due to the short growing season, given the late emergence of *Salix* and the fact that I terminated the experiment in June. In addition, the negative relationship between density and dry biomass in *Salix* could indicate intense intraspecific competition among individuals, possibly due to the limited space or depth for root growth of this tree species. Thus, this result may have been an artifact due to the pots in which I carried out the experiment.

In *Oenanthe*, I observed a similar decrease in dry biomass between years despite this species' early emergence. Since no negative relationship between density and biomass was found in this species, it is not clear what the cause of this reduction was. Possibly the long periods of rain in the winter and spring reduced the availability of required nutrients, limiting the growth of *Oenanthe*. Thus, the discrepancy in the performance of *Sidalcea* in the first and second year may be due to an increased sensitivity of *Sidalcea* seedlings to competition, differences in the nutrient availability, increased below-ground competition, or a combination of the above.

Despite differences in a variety of experimental factors such as target plant age (seedlings vs. adult plants), environmental conditions (tidal disturbance, rooting space), competing communities (two-species vs. multiple species), and possibly nutrient levels, the results of the field and pot experiment were similar in that neither result provided clear evidence that *Lythrum* had a lasting effect on the performance of the rare, native *Sidalcea*. Thus, neither result suggests that *Lythrum* has a more detrimental effect on the native *Sidalcea* than native species do.

### *Segregation of Lythrum and Sidalcea*

The low abundance of *Lythrum* in the well-drained area, and the early emergence of *Sidalcea* could be important factors in mitigating the effects of interspecific interactions between the two species. However, the result of the

removal experiment in the field, if indicative of the intensity of the interactions, does not suggest that a strong competitive relief occurs as a response to *Lythrum* removal. In the marsh, a mean of nine (maximum 63) *Lythrum* stems per m<sup>2</sup> were observed in areas with *Sidalcea*, a density, which corresponds to a mean of only 0.16 (maximum 1.1) *Lythrum* stems per pot surface area (Fig. 2-3). The abundance of *Lythrum* where it co-occurs with *Sidalcea* may not so high as to exert a significant, negative effect. In addition, interactions with other, native species may prevent a strong response of *Sidalcea*.

The early time of emergence likely allows *Sidalcea* to gain a competitive advantage by acquiring resources such as light and nutrients at a time when many of its competitors have not yet emerged. It is well known from weed research that time of emergence has a critical influence on crop yield (e.g. Odonovan 1992, Blackshaw 1993) due to interference by shading. In the case of *Sidalcea*, the advantage of early emergence may be crucial in determining its competitive ability given the improvement in the performance of the species in year 2 of the pot experiment.

#### *Transplant experiment*

The area into which *Lythrum* plants were transplanted did not affect plant shoot height, which suggests that the different drainage regimes and possibly differences in disturbance do not affect established plants in the short term. However, vegetation removal had a significant effect on shoot growth. The

smaller shoots in the removal treatment may have been due to the decreased protection from the tidal water movement. In fact, the shoots of experimental plants were frequently broken, apparently due to tidal disturbances. Differences in shoot development in the two areas over time were also due to shorter shoots in the well-drained area in June, but not later. However, the increased intensity of disturbance did not seem to affect shoot survival in the short term since the survival of individuals did not differ among treatments. Thus, the differences in shoot growth may not have long-term consequences, and my results suggest that the physical conditions in the East area are suitable for *Lythrum* growth and survival, and, at least in the short term, allow *Lythrum* to perform equally well as in the West area, where the density of *Lythrum* is higher. The observation that *Lythrum* is abundant in the waterlogged West area in which *Sidalcea* does not occur could indicate that *Lythrum*, unlike *Sidalcea*, is able to tolerate the stressful lack of oxygen.

#### *Lythrum seed availability*

The flush of *Lythrum* seedlings that appeared in artificially created openings in areas of *Sidalcea* growth in Ladner marsh indicate that it is not a general shortage of seeds in the soil that prevents the invader plant from reaching higher abundance there. Rather, the presence of established plants may prevent *Lythrum* seed germination.

Recruitment of *Lythrum* in the East area seems to be limited by the availability of gaps in the vegetation. This conclusion is supported by another study (Rachich and Reader 1999) in which seedlings of *Lythrum* only established after removal of reed canary grass in experimental plots. The authors concluded that disturbance is a requirement for wetland invasion of *Lythrum*. In Ladner marsh, natural disturbances are frequent and are caused by the water movement itself and debris and logs carried in at high tide. When the water recedes, the material is deposited and smothers the underlying vegetation.

### *Conclusions*

Based on the available evidence, I conclude that there is not enough evidence to suggest that *Lythrum* has a detrimental effect on the *Sidalcea* population in the short term. The invasion of *Lythrum* was largely completed by 1979, as shown by the statistically nonsignificant increase in abundance 20 years later, and the negative relationship between the two species in Ladner marsh may be due to differences in their realized ecological niches. In fact, the available evidence suggests that the two species differ in spatial and temporal dimensions: their time of emergence and their distribution within the marsh.

*Lythrum* seems to be able to persist only at low density in *Sidalcea* habitat, and the competitive effect of *Lythrum* does not appear severe as shown by the results of the removal experiment; however, this result could be due to spatial segregation between the species as a consequence of strong underlying

interactions. While the effect of *Lythrum* on *Sidalcea* seedling growth in year 1 of the pot experiment is indicative of such a severe, negative impact, *Sidalcea* seedling mortality in the *Lythrum* treatment was not higher than in the *Oenanthe* treatment.

Due to the comparatively low density of *Lythrum*, the presence of native species in areas with *Sidalcea*, and the differences in times of emergence, only a weak competitive effect was observed. Based on the results of my experiments, control methods for *Lythrum* in Ladner marsh do not seem necessary although I did not address all interactions, such as competition for pollinators. It is clear that the results of this study cannot be easily generalized to interactions in non-tidal environments because the distribution of *Lythrum* in Ladner marsh ultimately seems to be determined by the stressful physical conditions (levels of drainage and disturbance). In benign, non-tidal environments and with respect to other species, competition may be more intense.

#### *Conceptual model of wetland community organization*

My results can be interpreted in the context of an existing general model of vegetation zonation in wetlands. Keddy and collaborators proposed the so-called centrifugal organization model for wetland species distribution, which suggests that species have similar fundamental niches, but that a trade-off between the ability to exert competitive effects and to tolerate stress determines their occurrence (Wisheu and Keddy 1992, Keddy 2000). This model predicts

that highly competitive species will dominate core habitats with low disturbance and high fertility. Weaker competitors will be restricted to peripheral habitats with more stressful environmental conditions to which they are adapted. Thus, the limit of a species' distribution is determined by competition under favorable conditions and by the ability to tolerate harsh physical conditions under stressful conditions.

Fitting the results from my study into Keddy's centrifugal organization model, I propose the following conceptual model for the patterns of occurrence of *Lythrum* and *Sidalcea* in Ladner marsh: the well-drained and fertile conditions in areas where *Sidalcea* occurs are suitable for the growth of both species. Competitive interactions, particularly with clonal species, limit the germination of *Lythrum* seeds and prevent dominance of *Lythrum* in the well-drained areas of the marsh. Tidal disturbances create gaps in the vegetation necessary for *Lythrum* seed germination.

In the poorly drained areas of the marsh the stressful conditions do not allow *Sidalcea* to survive. However, *Lythrum* can occur here at high densities because it is adapted to the low soil oxygen levels. In Ladner marsh, *Lythrum* stems in waterlogged conditions develop an aerenchyma, a porous tissue at the base of the stems, which allows transportation of oxygen to the root system (Lempe et al. 2001).

My model agrees with J. P. Grime's observations in Britain that *Lythrum* "...appears to be excluded where disturbances are too frequent or too severe ... but it cannot persist where stable, productive conditions allow monopoly by clonal

herbs..." (Grime, J.P., <J.P.Grime@sheffield.ac.uk>, personal email, March 28, 2002). If the ecological profile of *Lythrum* in North America is comparable, it may be the presence of clonal species such as the sedge *Carex lyngbyei* Hornem. or the grass *Festuca arundinacea* Schreb., which are commonly associated with *Sidalcea* in Ladner marsh, that prevent the establishment of a high density of *Lythrum*.

Two predictions arise from this model that can be tested in the field. First, I predict that tidal disturbances and well-drained conditions allow the occurrence of clonal species, such sedges and grasses, in the habitat of *Sidalcea*. Consistent removal of these clonal species should promote an increase in the abundance of *Lythrum*.

Second, the observation that *Sidalcea*'s distribution is limited to the well-drained areas of the marsh suggests only poor adaptation to waterlogged soils. However, this could indicate competitive superiority in comparison with *Lythrum* because, according to Keddy's centrifugal organization model, there is a trade-off between a species' competitive ability and its ability to tolerate stress. In fact, the combination of many of *Sidalcea*'s traits, its early emergence in the spring, its perennial life-style and its comparatively great height and leaf-density may confer a superior competitive ability.

## CHAPTER THREE

# BIOLOGICAL CONTROL OF *LYTHRUM SALICARIA* IN THE LOWER MAINLAND OF BRITISH COLUMBIA

### **1. Introduction**

Introductions of insects for the biological control of invasive plants represent large-scale, ecological experiments. Although there are examples of biological control with spectacular success, the average success rate of biological control projects against weeds in the past has been low with less than one in six projects resulting in satisfactory control (Crawley 1989a, McFadyen 2000). In most cases the biological control agents did not establish or failed to exert enough predation pressure on the weed to lower its abundance (Sheppard 1992).

The success of a biological control program depends on both sufficiently high densities of the biocontrol agent and its ability to inflict debilitating or lethal damage. Since host plants are usually abundant at sites into which the biological control agents are introduced, local biotic and abiotic factors and their interaction ultimately determine the abundance of the control agent at each site. Among the factors invoked as causes of variation in herbivore density, are biotic interference with predators, plant quality, and habitat stability.

Local parasitoids and predators are frequently suggested as the causes of failure in biological control projects (Goeden and Louda 1976), and ants are often on the list of

potential culprits (Cullen and Snowball 1979, Crawley 1989a, Cullen 1995).

Alternatively, host plant quality can play a decisive role in determining control agent abundance. An agent can be successful in one environment, but not in another due to the poor quality of the host plant at one site that does not allow population growth of the control agent (e.g., Room 1990). The establishment and success of a biological control agent is also influenced by habitat stability. Stable habitats such as forests are associated with higher rates of establishment of control agents than disturbed habitats such as agricultural fields (Hall and Ehler 1979, Cameron et al. 1993).

Even when establishment is successful and conditions are optimal, some natural enemies are ineffective and cannot control their host (Cullen and Snowball 1979). Only a control agent that is able to inflict damage to the life history stage that is critical to regulating the host plant population (the stage after which compensation cannot occur) can be successful in reducing host abundance. For example, an insect that attacks flowers and developing seeds will not affect plant density if plant recruitment is not limited by seed availability, but by the availability of microsites (Crawley and Gillman 1989, Myers and Risley 2000). However, when repeated, severe defoliation depletes the plant's reserves, the attacked plants will eventually die and a decline in plant density can result. Thus, if an insect has reached high density and is attacking a critical life history stage, a reduced plant density is expected to occur in the long term.

The effects of herbivory on plant performance are commonly proportional to the amount of tissue lost (Hendrix 1988), such that even low-level herbivory can affect individuals. However, in many plant species, a threshold level of defoliation exists below which individual performance is not significantly affected due to regrowth following herbivory (Hendrix 1988). The existence of stored food reserves and axillary buds

facilitates this response (Hendrix 1988). Therefore, in such plant species, weak herbivory is unlikely to affect plant density.

The biological control of purple loosestrife, *Lythrum salicaria* L. (Lythraceae), in North American wetlands by European, leaf feeding, chrysomelid beetles is a recent example of repeated biological control success (reviewed in Blossey et al. 2001b). In Manitoba, Lindgren (2003) observed a dramatic reduction of *Lythrum* stem density four years after the introduction of the control agent, and after six years grazing by the beetle resulted in local elimination of all *Lythrum* stems. And yet, the beetles have not successfully established at all North American sites nor do they control the abundance of their host plants in all locations. In the Lower Mainland of British Columbia, *Lythrum* has infested wetlands including sites along the Fraser River that are strongly influenced by the tides. Here only one control agent, *Galerucella californiensis* L. (Coleoptera: Chrysomelidae) has been widely introduced during the past ten years.

In this study, I ask three main questions.

**Question 1: What is the spatial and temporal variability in beetle feeding damage among release sites?**

**Question 2: What factor(s) can explain the variability in *G. californiensis* feeding damage among sites, if it exists?**

In addressing this question I differentiate between tidal and non-tidal environments. In tidal environments, the disturbances associated with the water movement could be of overwhelming importance in determining beetle establishment and abundance. Since the larvae of *G. californiensis* feed externally on the leaves, they may be particularly vulnerable to dislocation by the disturbances associated with high

tides. Thus, I predict (i) that disturbances associated with the water movement negatively affect beetle survival at tidal sites.

At non-tidal sites, interference with general predators such as lady beetles and lacewing larvae has been suggested to affect the population growth of *G. californiensis* (Nechols et al. 1996, Wiebe and Obrycki 2002). If predators influence beetle density, (ii) site differences in predation on the control agent (predation hypothesis), but not in plant quality (plant quality hypothesis), will be good predictors of plant damage in a given year. (iii) If predators are crawling rather than flying, predation rates of eggs will be lower on stems in the water. If site differences in predator abundance rather than in predator attack rates (cf. Holling 1959) are causing different levels of predation, (iv) the predation rates at the different sites will be related to the densities of crawling predators.

### **Question 3: What is the effect of herbivory on the performance of *Lythrum*?**

I predict that (v) herbivory will affect *Lythrum* vegetative and reproductive performance in the short term. (vi) *Lythrum* density, however, will only decrease after suffering consistent, intense herbivory for four years if the beetle attacks a critical life-history stage of the weed. Given extensive food reserves and the existence of axillary buds in *Lythrum*, (vii) low-level herbivory, even when consistent over four years, will have little effect on *Lythrum* stem density.

Using a combination of long-term monitoring and short-term experiments, as recommended by McEvoy et al. (1991), I have studied the establishment and effectiveness of this beetle. A regional survey over a period of four years allowed me to measure spatial and temporal variation in beetle feeding damage (Question 1), and to detect the immediate and cumulative effect of beetle herbivory on *Lythrum* performance (Question 3). Field and laboratory experiments have been used to investigate the roles

of disturbance by tidal water movement, predation and plant quality on beetle density and behaviour (Question 2).

## **2. Methods**

### **2.1 Study system**

#### *Galerucella californiensis*

To date, four specific natural enemies that are associated with *Lythrum* in its natural range have been introduced into North America in an attempt to control the weed: two species of leaf feeding beetles, *Galerucella californiensis* L. and *Galerucella pusilla* (Duft.), a root weevil, *Hylobius transversovittatus* Goeze, and a flower feeding weevil, *Nanophyes marmoratus* Goeze (Julien and Griffiths 1998). In the Lower Mainland of British Columbia, mainly one species, *G. californiensis*, has been introduced beginning in 1993 (Table 3-1). There is an extensive literature on the biology, ecology and taxonomy of this species (Blossey 1991, Hight and Drea 1991, Malecki et al. 1993, Manguin et al. 1993, Blossey 1995, Medina 1995, Nechols et al. 1996, Blossey and Schat 1997, McAvoy et al. 1997, Sebolt and Landis 2002, Velarde et al. 2002). I report here only briefly on the species' life history.

The adults emerge from the soil in early to mid-May and feed on leaf tissue of the host plant. Following a period of seven to ten days, the main oviposition period occurs during May and June with reduced egg production until the end of July. Each female

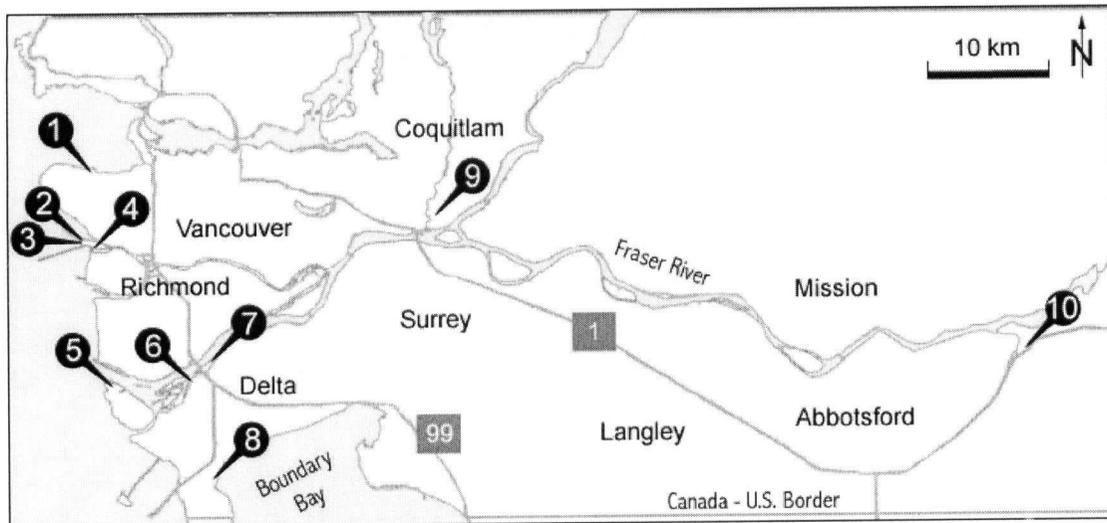
**Table 3-1** Introductions of *Galerucella* sp. to the Lower Mainland of British Columbia. \* indicates failed control agent introduction; ? unknown information.

Site	Coordinates	Year of release	Number released	Species	Site Conditions
Boundary Bay Park	49°01.40'N, 123°03.17'W	1995	475	90 % <i>G. californiensis</i>	Non-tidal
Boundary Bay Park	49°01.40'N, 123°03.17'W	1996	216	10 % <i>G. pusilla</i> <i>G. californiensis</i>	Non-tidal
Chilliwack	49°07.77'N, 122°05.00'W	1997	200	<i>G. californiensis</i>	Non-tidal
Colony Farm	49°14.27'N, 122°47.46'W	1997	300	<i>G. californiensis</i>	Non-tidal
Deas Island	49°06.59'N, 123°03.45'W	1997*	310	<i>G. californiensis</i>	Tidal
Iona marshes	49°13.2'N, 123°12.7'W	1993*	?	<i>G. californiensis</i>	Non-tidal
Iona North marsh	49°13.2'N, 123°12.7'W	1997	300	<i>G. californiensis</i>	Non-tidal
Iona South marsh	49°13.2'N, 123°12.7'W	1997	300	<i>G. californiensis</i>	Non-tidal
Jericho Beach Park	49°16.30'N, 123°11.99'W	1993	38	<i>G. californiensis</i>	Non-tidal
Jericho Beach Park	49°16.30'N, 123°11.99'W	1997	300	<i>G. californiensis</i>	Non-tidal
Ladner marsh	49°07.54'N, 123°05.05'W	1999*	500	<i>G. californiensis</i>	Tidal
MacDonald slough	49°12.62'N, 123°12.18'W	1997*	600	<i>G. californiensis</i>	Tidal
Westham Island	49°06.53'N, 123°10.99'W	1993*	270	<i>G. californiensis</i>	Non-tidal
Westham Island	49°06.53'N, 123°10.99'W	1997	100 -150	<i>G. californiensis</i>	Non-tidal

can lay up to 500 eggs in small batches of two to ten eggs on the stems, leaves and the leaf axils of *Lythrum*. Small neonate larvae appear in early June and crawl to the top leaves to feed. The larvae molt three times with more than 80 % of the larval growth and damage occurring in the last larval instar (Loos and Ragsdale 1998). Developing larvae move to the underside of leaves and feed extensively on leaf and stem tissue. After two to three weeks the larvae move into the soil underneath the host plant and form a prepupa that develops into a pupa. The adults of the next generation emerge in July, two to three weeks after the larvae have entered the soil. The adults continue feeding until October before seeking overwintering sites in the soil. A low density of new egg masses found in August 1999 at two of the monitoring sites suggest that this species is partially bivoltine in southwestern British Columbia.

## **2.2 Study sites**

Monitoring and experiments were conducted at ten sites in the Lower Mainland of British Columbia (Fig. 3-1), at which *Galerucella* spp. beetles had been released by Roy Cranston, the B.C. Ministry of Agriculture, Food and Fisheries, in collaboration with Dr. Judith Myers from the University of British Columbia from 1993 to 1999. Table 3-1 summarizes information about the sites and the released beetles. General trends of beetle populations are described for the study sites below.



**Fig. 3-1** Map showing experimental sites. ❶ Jericho Beach Park; ❷ Iona North Marsh; ❸ Iona South Marsh; ❹ MacDonald Beach; ❺ Westham Island; ❻ Ladner Marsh; ❼ Deas Island; ❽ Boundary Bay Park; ❾ Colony Farm; ❿ Chilliwack Roadside (Yale Road exit); 1, 99 Highways.

### *Iona North Marsh and Iona South Marsh*

The Iona North and Iona South sites are situated along the Fraser River just north of the Vancouver International Airport. Both are non-tidal, artificial freshwater ponds created in 1990 (Mooney 1990) and are similar with respect to size and vegetation. A continuous, approximately 400 m long ring of *Lythrum* surrounds both marshes. At their closest point, a 50m-wide strip of land separates the ponds. Native wetland vegetation is limited at both sites with mainly *Typha latifolia* L. (cattail) stands remaining between *Lythrum* plants. In 1994, 178 individuals of *Hylobius transversovittatus* were released at Iona, but this species was never recovered. The first successful release of *G. californiensis* beetles was in 1997. In 1999, a small, localized outbreak of beetles (i.e. complete defoliation of *Lythrum* plants) was observed at the North marsh. As of 2000, the *Lythrum* surrounding half of the pond showed considerable feeding damage with complete loss of *Lythrum* foliage and suppression of flowering. However, following beetle dispersal in the fall of 2000 after the food source was depleted locally, reduced beetle activity occurred in 2001 with a lower overall impact. The area with defoliated *Lythrum* comprised less than 200m<sup>2</sup>. In 2002, the outbreak area expanded again to include the entire *Lythrum* population surrounding the North marsh. In contrast with the North site, only individual plants were completely defoliated at the South marsh, and the overall impact appeared small in all years.

### *Jericho Beach Park*

In Jericho Beach Park *Lythrum* is distributed evenly in a small pond (approximately 0.25 ha) that fills with rainwater each winter and dries during the summer months.

The two dominant plant species are reed canary grass, *Phalaris arundinacea* L., and *T. latifolia*. In 1993, 38 *G. californiensis* obtained from reared stocks at the Agriculture and Agrifood Canada Lethbridge Research Centre were released, and observations in 1994 / 95 showed that beetles had established at the site. In 1997, 300 more *G. californiensis* beetles, obtained from Ontario, were released. High densities of beetle larvae during the entire observation period caused considerable loss of foliage in the *Lythrum* plants. While no inflorescences were recorded in the monitored quadrats in 1999 - 2001, individual flowering stems were observed nearby. Hand pulling of *Lythrum* was carried out at this site prior to 1998, and in 1998, *Lythrum* flowering stalks were cut at the site to avoid *Lythrum* seed set.

### *Westham Island*

This site is in a nature reserve on Westham Island at the mouth of the south arm of the Fraser River, approximately 20km south of Vancouver. The monitoring site in "field 9" at the Canadian Wildlife Service land consists mainly of reed canary grass and patches of *Lythrum*. In the winter, rainwater creates a shallow pond, which dries in the summer. In 1993, an initial release of 270 *G. californiensis* beetles reared from stocks at the Agriculture and Agrifood Canada Lethbridge Research Centre was unsuccessful. A population established initially, but declined afterwards. In 1997 *G. californiensis* was

established at this site by releasing just over one hundred beetles from Ontario. Beetle density at Westham increased gradually from 1999 to 2002. A localized beetle outbreak in 2001 and 2002 over a 0.5-ha area with scattered *Lythrum* plants resulted in complete removal of foliage and suppression of flowering.

#### *Colony Farm Regional Park*

The 262 ha Colony Farm Regional Park is located along the Coquitlam River, approximately 60km east of Vancouver. *Lythrum* distribution is patchy, but increasing according to park officials. Large areas are covered by reed canary grass. Where the ground is covered by water until late in the spring, low growing buttercups, *Ranunculus* cf. *repens* L., form a lawn-like ground cover.

In 1997 *G. californiensis* beetles, offspring of parents collected earlier in the summer from Ontario, were released in the "Wilson farm" area. Beetles were observed at least 100m from the original release site in 1999, but feeding damage to *Lythrum* remained low until 2002. A local beetle outbreak in 2002 led to complete defoliation and elimination of flowering in the monitored patches. In 2001, an infestation of spittle bugs (Cercopidae) was associated with stunted and branched growth of *Lythrum* shoots.

#### *Boundary Bay Regional Park*

This 182 ha-park is situated on the shore of Boundary Bay, 30 km south of Vancouver. *Lythrum* occurs along ditches and in distinct, large patches. *Lythrum* patches are surrounded by low growing grass species. The ground is under water in the spring, but dries during the summer. Spittle bugs were commonly observed on *Lythrum*

at this site in the spring of each year, and *Lythrum* stems were small and highly branched. In 1995, *Galerucella* spp. beetles were successfully released at this site, 10 % of which were *G. pusilla*. It is not known if both chrysomelid beetles were established and are now present in the field, since their similarity in appearance and ecology makes it difficult to distinguish them (Manguin et al. 1993). Beetle density increased over the four-year monitoring period, but outbreak levels were not reached. In 2002, only individual plants showed partial defoliation.

#### *Chilliwack Roadside*

This small site (0.05 ha) is a wet habitat fed by runoff water and is located approximately 100 km east of Vancouver, at the Yale road exit on Highway 1. A third of the area of the site is submerged all summer, while the remaining area remains moist but without standing water. In 1997, when *G. californiensis* beetles were released, the vegetation consisted mainly of *Lythrum*, reed canary grass and cattail. By 1998, the density of control agents was high enough to defoliate the *Lythrum* plants completely. Beetle outbreaks were sustained, and *Lythrum* flowering was eliminated in all four years. By 1999, the beetles had dispersed to a small *Lythrum* infestation at a distance of approximately 200 m from the release site.

#### *Deas Island, MacDonald Slough and Ladner Marsh*

These three sites are located along the Fraser River and are strongly influenced by the tides. Due to the Fraser River influx the water is mostly fresh. At all sites, the vegetation is submersed at high tide. The vegetation consists of dense and uniform populations of

*Lythrum*, intermixed with other native and nonnative plants. In 1997, *G. californiensis* were released at Deas and at MacDonald slough. Until 1999 individual beetles occurred in the upper-most area of both sites, that is less frequently flooded, and caused negligible feeding damage. However, no beetles were observed in lower areas that are flooded with each high tide. In 1999, *G. californiensis* was released at Ladner marsh. In 2000, individual beetles and some feeding damage were observed at the release site. In 2001 *G. californiensis* could no longer be found at any of the sites.

### **2.3 Monitoring procedure**

Except for MacDonald slough and Iona North Marsh, all sites were monitored at least once a year, in late summer, from 1999 to 2002 to determine vegetative and reproductive performance of *Lythrum* and to estimate *G. californiensis* feeding damage. In 2000, the sites were visited once a month from May to August. Monitoring involved placing ten to 15 1 m<sup>2</sup> quadrats along an arbitrarily chosen transect in the *Lythrum* infestation (Blossey and Skinner 2000). Plots with fewer than five *Lythrum* stems were not considered. Beetle density at each site was estimated by counting the different life-history stages (eggs, larvae, adults) separately during one minute for each quadrat.

The plant feeding damage was determined at the end of July in the following way: one of six damage categories based on the estimated percentage removal of foliage, A: 0 %, B: ≤ 5 %, C: 6 - 25%, D: 26 – 50 %, E: 51 – 75 %, F: 76 – 100 %, were subjectively assigned to the *Lythrum* stems in each quadrat. The overall feeding damage at a site was computed by averaging the midpoints of all damage categories over all sample quadrats. Therefore, the theoretical maximum feeding damage was 88 %.

I estimated *Lythrum* abundance by recording the number of stems per square meter and measuring the height of the five tallest stems. *Lythrum* stem densities recorded during all four monitoring events were averaged.

To estimate *Lythrum* biomass in August 2000, 30 plant shoots, the three tallest and the three shortest *Lythrum* stems in each of five one square-meter plots, were cut at their base and dried at 80 °C for 48 hours. Average *Lythrum* plant biomass per square meter for the  $j$ th site,  $A_j$ , was computed as:

$$A_j = \frac{d_j}{30} \sum_{i=1}^n \frac{a_{ij}}{n_j}$$

where  $d_j$  = combined dry biomass of the three tallest and the three shortest stems sampled in each of five quadrats at the  $j$ th site,  $a_{ij}$  = number of *Lythrum* stems in the  $i$ th quadrat,  $n_j$  = number of quadrats sampled at the  $j$ th site.

*Lythrum* reproductive performance was determined by taking the following measures of the five tallest stems in each quadrat: the length of the inflorescences, the density of flower buds on the inflorescences (number per five cm) and the number of inflorescences per stem. In addition, the total number of inflorescences per square meter was counted. For each site, I computed a flower index,  $F_j$ , as an overall measure of *Lythrum* reproductive output in August 2000. The index was computed as:

$$F_j = \frac{\sum_{i=1}^n l_{ij} m_{ij} p_{ij}}{n_j}$$

where  $l_{ij}$  = average number of flower buds per cm of inflorescence of the five tallest *Lythrum* stems in the  $i$ th quadrat,  $m_{ij}$  = average length in cm of the inflorescences of the five tallest *Lythrum* stems in the  $i$ th quadrat,  $p_{ij}$  = total number of inflorescences in the  $i$ th quadrat, and  $n_j$  = number of quadrats sampled at the  $j$ th site.

## **2.4 Experiments**

### *Beetle survival in tidal areas*

To test the hypothesis that disturbance associated with the water movement and the flooding conditions may cause failure of beetle establishment in tidal areas I carried out a series of laboratory and field experiments at the egg, larval and pupal stages.

#### Eggs

To determine if the tidal activity washes away egg masses of *G. californiensis*, ten plants with egg masses were selected from Iona of *Lythrum* in mid-May 2002. The plants were divided in two homogeneous groups with regard to their height and egg load. On each plant, I selected six to ten egg masses in regular height intervals and marked them with a small dot of Liquid Paper<sup>®</sup> just above the egg mass. Each group of plants was then transplanted into one of the two tidal areas, Deas and MacDonald slough. Plants were placed at a distance of 5 m in a straight line and were inspected after 15 days for the presence of the marked egg masses and any larvae.

Five egg masses on each of 16 stems were marked and monitored simultaneously, but for seven days only, at Iona South marsh, a non-tidal site. Differences in disappearance rates between the tidal sites and the non-tidal site were compared using a Mann-Whitney U test. I tested if the presence of larvae, which hatched from the eggs on the experimental stems, differed between tidal sites with the Fisher exact test, since the number of larvae was small or zero on most plants.

## Larvae

I inoculated twelve individual *Lythrum* stems with five beetle larvae each in late afternoon of June 14, 2001, at MacDonald slough and determined the number of remaining larvae the next morning, after a high tide-event. Two treatments were applied: second and third instar larvae were either set on the tips of tall plants, which were not submerged during the high tide, or on small, completely submerged plants. Maximum tide level relative to the *Lythrum* stems was recorded during the high-tide event to ensure discriminating treatments to the larvae on tall and small stems, respectively. The data were analyzed using a Mann-Whitney U test.

## Pupae

In a laboratory experiment I examined the ability of the beetles to pupate when their host plant is surrounded by water, as can be the case under tidal conditions. One small *Lythrum* plant was potted in each of ten 20 cm tall plastic containers. Each plant received five third-instar larvae close to pupation on June 28, 2001. The containers were covered with clear plastic to prevent the beetles escaping. Two treatments were applied to five replicates each. In the control treatment, the water level in the pots was kept only 5 cm high, well below the natural pupation depth of 1-1.5 cm below the soil surface to generate well-watered but drained conditions. The flooding treatment involved filling the containers completely with water after the larvae were allowed to move into the soil to pupate. The containers were left at room temperature. After a ten-day period, I recorded the number of adult beetles that had emerged. Using a Fisher exact test, I tested whether beetle survival depended on flooding condition.

## *Variation in predation, predators and food plant quality among sites*

The non-tidal study areas Iona North marsh, Iona South marsh, Jericho Beach Park and Westham were selected as experimental sites, since they had shown varying *Lythrum* feeding damage in 2000. I limited the number of experimental sites to four to ensure that they could all be visited the same day given to the amount of labor and traveling time involved. Beetle feeding damage, predation on the beetles, and plant quality, measured as the weight of larvae protected from predators, were determined at each site. Beetle plant feeding damage was determined as described in the monitoring procedure. The average feeding damage per site was then correlated with predation rates on eggs, larvae and pupae and measures of plant quality.

### Egg predation

Egg predation was assessed in mid-May. First, I determined the height distribution of the egg masses at each site from ten arbitrarily selected *Lythrum* stems. I then selected one experimental egg mass on each of 50 arbitrarily chosen *Lythrum* stems such that the height distribution of the experimental egg masses reflected the natural distribution. The position of each egg mass (in five-cm intervals from the ground) and the number of eggs in the egg masses were recorded. Each egg mass was marked by placing a small dot of Liquid Paper<sup>®</sup> correction fluid just above it (Nechols et al. 1996). After a seven-day period, I recorded the condition of the eggs. Shrunken eggs, missing eggs, and eggs which had been opened were considered to be preyed upon. The predation rate for each site was computed as the average predation rate of all 50 egg masses.

## Influence of water level on egg predation

To determine if standing water had a protective influence on egg predation, I carried out an experiment at Iona South marsh. The site was chosen because individual plants in the water had shown high defoliation levels in 2001, but not plants on land. In 2002, I measured the predation rates of egg masses on *Lythrum* stems surrounded by water or not surrounded by water. A total of 15 individual *Lythrum* plants were selected in mid-May, seven in standing water, eight on land. Five egg masses were chosen at regular intervals on each stem and marked with a small dot of Liquid Paper<sup>®</sup> just above the egg mass. The number of eggs of each egg mass was recorded. After one week, I recorded if predation (yes or no) had occurred to each of the egg masses. The mean predation levels per plant stem were compared using a Mann-Whitney U test.

## Predation on larvae

To estimate predation on larvae, larvae were exposed to predators at each of the four experimental sites. To assure equal plant quality, the *Lythrum* plants to receive the experimental larvae were grown in the greenhouse from stem cuttings. Four weeks before the experiment started, *Lythrum* shoots were harvested at Iona South marsh and transferred to the UBC greenhouse immediately. Here, all except three leaves at the shoot tips were removed, and the shoots were planted singly in plant pots filled with mineral soil and put in the mist bed to grow roots. Water sprinklers and heating cables for bottom heat maintain a humid (80 % relative humidity) and warm (25°C) climate in the mist bed that encourages plant growth.

I used a total of 20 plants per site. The plants were paired to produce ten sets: one of the plants was selected randomly and received ten second-instar larvae; the other plant served as a control for immigrating larvae because larvae disperse from plants when severe defoliation leads to food shortage (personal observations). The control plant was placed at a distance of 20 cm from the experimental plant. Each pair of plants was considered a replicate and was at least one meter from other replicates. The potted plants were placed in the soil such that the containers were completely buried. Water for the plants was provided when necessary, usually daily. After a six-day-period, the plants were recovered, and the larvae were counted. Missing larvae were considered killed by predators. The number of surviving larvae was determined by computing the difference in the number of larvae between each pair of plants to correct for larval immigration. To compute the mean number of surviving larvae at each site, I averaged the number of surviving larvae of all plant pairs.

#### Predation on pupae

To measure predation on pupae, I buried 20 sets of four pupae 1 - 1.5 cm under the soil surface, in their natural pupation environment, at each of the four experimental sites. To facilitate recovery of the pupae, I placed them on a 10 cm x 10 cm piece of mesh screen and marked their location with a small wooden stick. The number of recovered pupae was recorded after a week, missing pupae were considered preyed upon.

## Densities of crawling predators and levels of predation

During the time when the experiments were in place I monitored the presence and abundance of crawling predators at the four experimental sites using pitfall traps. Five traps were placed at a distance of five meters from each other. The pitfall traps consisted of nested, clear plastic cups half-filled with propyl glycol. The inner sampling cups measured six cm in diameter and were sunk into the ground such that the trap lip was flush with the substrate. Square covers of transparent plastic were held five to six cm above the traps with nails in each corner to prevent influx of rainwater, but to allow arthropods unrestricted access to the traps. The traps were put out at the same locations until the end of July and emptied biweekly. When the samples were collected, new preservative was added to the traps. The invertebrate predators in the traps were stored in 70 % ethanol and sorted into three groups: ants, beetles, and spiders. The small number of other invertebrates was not considered in the subsequent analyses.

To determine if invertebrates present in the pitfall traps were related to the observed predation rates, I correlated the total number of individuals of the different invertebrate groups found in the five traps (beetles, ants, spiders) with the computed predation rates in eggs, larvae and pupae. To meet the assumptions of correlation analysis, square root transformation was used to transform the count variables, and arcsine transformation was applied to the predation rates.

## Plant quality

Plant quality was assessed indirectly by measuring the weight and survival of caged larvae after feeding on individual plants. I caged ten arbitrarily selected plants in

early May to prevent oviposition by adult beetles. When larvae were available, I inoculated each plant with ten first instar larvae from nearby plants. Since most of the larval growth takes place in the third instar, initial larval weight differences were considered insignificant and were not recorded. Crawling predators were prevented from accessing the plants by applying Stickem<sup>®</sup> (Phero Tech Inc., Richmond B.C.) to the base of the cages and the base of the plant stems. After 13 days the larvae were recovered, counted and weighed.

#### Data analysis

Each of the four variables examined was correlated individually with the percentage plant damage at each site using Pearson linear correlation, since data limitations prevented the use of multiple regression techniques. To assess if pupal predation influenced adult survival, pupal predation in the fall of 2001 was correlated with adult density in the spring of 2002.

All variables were transformed to meet the assumptions of linear correlation analysis. Angular transformation was used to transform proportions (percentage plant damage, predation rate of eggs), square root transformation was applied to the count variables (surviving larvae, surviving pupae), and larval weights were log transformed.

#### *Impact of beetle feeding on individual host plant performance*

Using the monitoring data obtained according to the procedure outlined earlier, I carried out a series of simple linear correlation analyses to evaluate the impact of the control agents on *Lythrum* in 2000. Only data from the sites where control agents were

present were used for this analysis. Feeding damage recorded in July 2000 was correlated separately with each of several variables that indicate the reproductive and vegetative performance of the monitored *Lythrum* populations described above.

Due to variability in local environmental conditions, *Lythrum* performance likely varied among different sites before the introduction of the control agent. This initial variation would need to be taken into account to estimate the effect of the control agent with more precision. However, data on the performance of *Lythrum* before the control agent was introduced are insufficient to include in the analysis. To estimate the variability of the performance indicators independent of control agent activity, I analyzed data from three tidal sites where no feeding occurred and used the among-site variability as a reference. If *Lythrum* performance at sites lacking beetles does not vary, then variation at sites with beetles is likely due to beetle feeding damage.

Measures of *Lythrum* performance among the three tidal sites was compared using analyses of variance, Welch analyses of variance (when the assumption of equal variance was not met) and non-parametric analyses of variance (when the assumption of normality was not met) on all variables tested for effects of control agent feeding. Count variables were square root transformed (the number of flower buds, the number of inflorescences, the flower index) and length- and height-measurements were log-transformed to meet the assumptions of the parametric statistical tests. Site was considered a random factor, and thus, no post-hoc tests were carried out when significant differences were found among the tidal sites.

## *Impact of beetle feeding on host plant abundance*

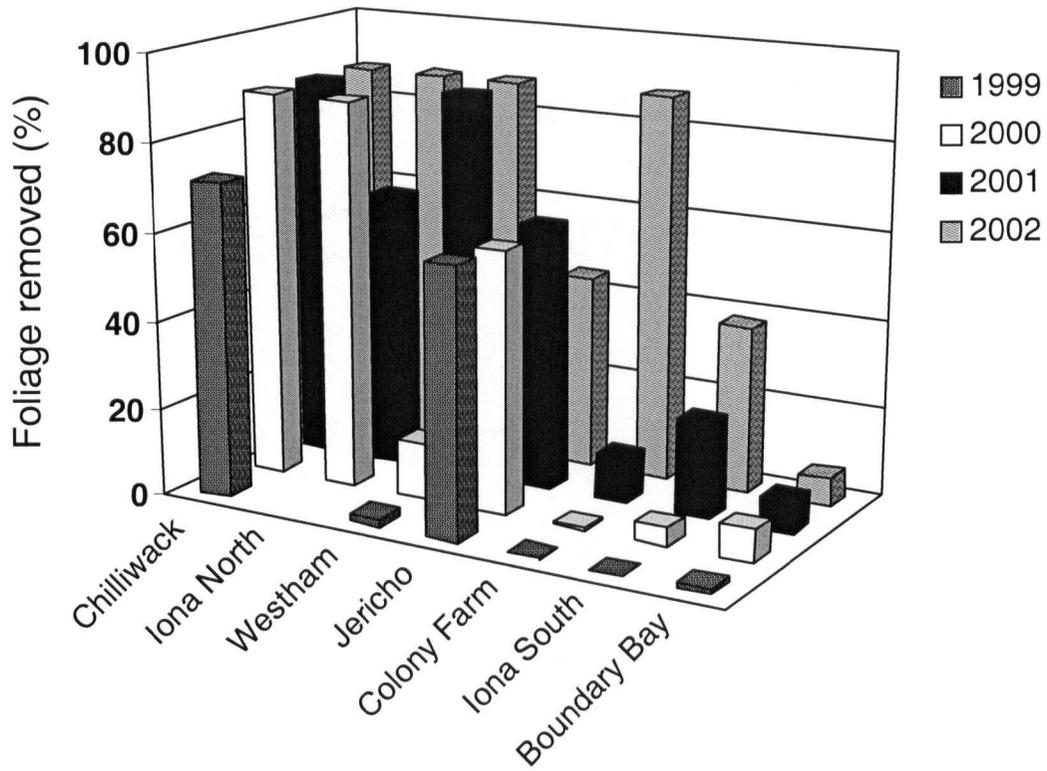
Using the monitoring data, I examined the combined impact of four years of herbivore feeding on the plant population density at all six non-tidal sites. Since the feeding damage in July was not recorded in all years and at all sites, *Lythrum* flower bud density, which was a sensitive measure of feeding damage (see Results), was used as a surrogate. Using Spearman rank correlation analysis, I examined the relationship between flower bud densities and the percentage change in stem densities between 1999 and 2002 at each site. To compare my results to a control, I also analyzed the changes in stem density at the three tidal sites during the same time period, using two-sample tests.

The Chilliwack site was the only site that had experienced consistent and complete defoliation during the four-year observation period. I evaluated the change in *Lythrum* stem density at this site using a Mann-Whitney U test.

### **3. Results**

#### *Variation in beetle feeding damage*

Feeding damage to *Lythrum* varied in space and time (Fig. 3-2), apparently reflecting differences in *G. californiensis* beetle densities. The beetles failed to establish at any of three tidal sites. The number of sites where beetles occurred at sufficient density to cause complete plant defoliation increased from two in 1999 to four in 2002.



**Fig. 3-2** Percentage foliage removed in populations of *Lythrum salicaria* from 1999 to 2002. Tidal areas, where the control agent failed to establish, are not shown. Error bars omitted for clarity.

A rapid increase in feeding damage, from low feeding damage to complete defoliation, can occur within a year (Westham, Colony Farm). Once outbreak densities were reached, high *Lythrum* defoliation levels were sustained in the following years. Consistently low herbivory levels were found only at Boundary Bay Park. Defoliation levels increased at all other non-tidal sites over the monitoring period.

### *Beetle survival in tidal areas*

#### Eggs

Although I expected that the disturbance associated with the tidal water movement would increase the loss of eggs from plants, this prediction was not supported. On average,  $12 \pm 15\%$  and  $19 \pm 17\%$  of all marked egg masses disappeared at Deas and MacDonald slough, respectively. Since these numbers also include egg mass loss due to predation and larval hatching, after which the remains of the eggs may disintegrate, the loss of egg masses solely due to tidal activity is smaller. One or more larvae were found on two of the plants from Deas and on all plants at MacDonald, indicating that at least some of the eggs remained viable after a two-week exposure to water. At the Iona South marsh,  $15 \pm 9\%$  of the egg masses disappeared during a one-week period only. The two tidal sites did not differ with regard to either the loss of egg masses ( $\chi^2 = 1.99$ ,  $P = 0.15$ ) or the presence of larvae (Fisher exact test,  $P = 0.18$ ). When the data from both tidal sites were pooled, the loss of egg masses at the tidal sites did not differ from the loss at the non-tidal site ( $Z = 0.19$ ,  $P = 0.85$ ).

## Larvae

Survival of larvae in tidal areas was related to plant height. In the field experiment, the tide in the night of June 14 was so high as to completely submerge the short *Lythrum* plants, but left the upper parts of the tall plants uncovered. Significantly more larvae disappeared from the short than from the tall stems ( $Z = 2.49$ ,  $P = 0.013$ ) during the high-tide event. Starting with five larvae,  $4.5 \pm 0.34$  larvae were recovered from the tall plants, on average, compared with only  $2.2 \pm 0.54$  from the short plants.

## Pupae

In the laboratory experiment, an average of  $3.6 \pm 0.51$  adult beetles developed from five larvae that were left to pupate in the control treatment. No adult beetles were observed in the flooding treatment, indicating a significant difference in pupal survival between the flooding and control treatment (Fisher exact test,  $P = 0.008$ ).

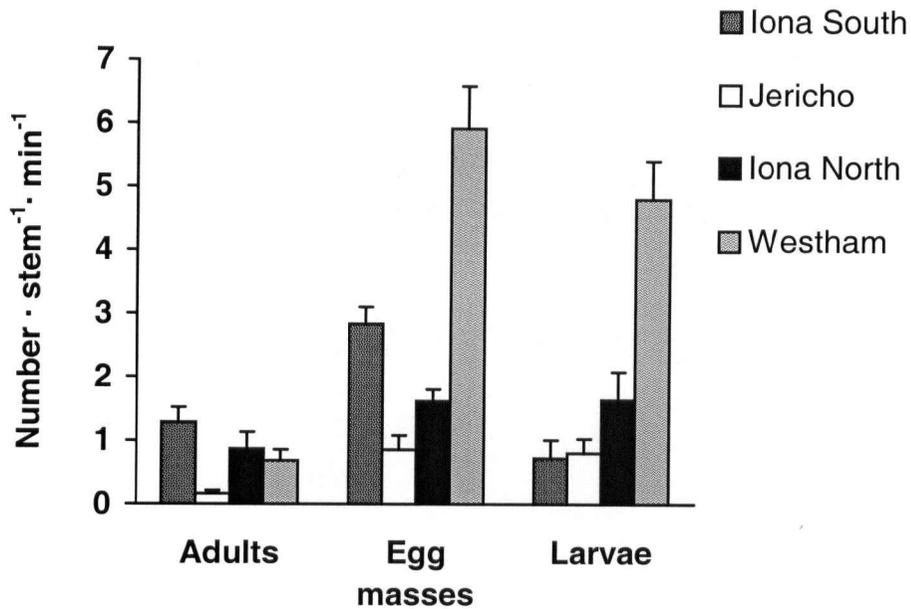
### *Variation in predation, predators and plant quality among sites*

#### Beetle density and feeding damage

Average *Lythrum* feeding damage at the four experimental sites ranged from a low of 22 % at Iona South to a high of 88 % at Westham (Table 3-2). Beetle density was highly variable between sites. On a per stem basis, adult beetle density was highest at Iona South marsh, the site with the lowest feeding damage, with 1.3 beetles observed per stem and per minute in May of 2001 (Fig. 3-3). This was due to dispersal of adult

**Table 3-2** *Lythrum* feeding damage (proportion foliage removed), *Galerucella californiensis* egg, larval and pupal predation levels (measured as the proportion of eggs, larvae and pupae preyed upon, respectively) and plant quality (measured as larval weight in  $10^{-6}$  g and the number of larvae recovered out of ten larvae, respectively) at each of four experimental sites in 2001. Experimental methods and results of the statistical tests are given in the text. Values are means  $\pm$  SEM.

	Iona South marsh	Jericho	Iona North marsh	Westham
Feeding damage	0.22 $\pm$ 0.06	0.61 $\pm$ 0.10	0.63 $\pm$ 0.05	0.88 $\pm$ 0.00
Egg predation	0.60 $\pm$ 0.06	0.26 $\pm$ 0.05	0.34 $\pm$ 0.06	0.07 $\pm$ 0.03
Larval predation	0.46 $\pm$ 0.07	0.22 $\pm$ 0.05	0.34 $\pm$ 0.12	0.30 $\pm$ 0.07
Pupal predation	0.25 $\pm$ 0.06	0.23 $\pm$ 0.06	0.11 $\pm$ 0.05	0.45 $\pm$ 0.10
Plant quality (larval weight)	3494 $\pm$ 501	3369 $\pm$ 385	4269 $\pm$ 348	2365 $\pm$ 270
Plant quality (larval survival)	9.30 $\pm$ 0.33	6.80 $\pm$ 0.80	9.00 $\pm$ 0.33	9.20 $\pm$ 0.29



**Fig. 3-3** Number (+ SEM) of adults, egg masses and larvae of *Galerucella* observed per *Lythrum* stem and per minute at four experimental sites in 2001. Adult and egg mass densities were recorded in mid-May, larval density in mid-June. Individuals of each life history stage were counted during one minute in a 0.25m<sup>2</sup> quadrat.

beetles from the nearby Iona North site. However, relative egg mass and larval densities were highest at Westham with 5.9 egg masses and 4.8 larvae observed per stem and per minute, respectively, but considerably lower at the other sites.

### Egg predation

Egg predation rates were highly variable, ranging from a low of 7 % at Westham to a high of 60 % at Iona South to (Table 3-2). Plant damage varied significantly with the level of predation on eggs ( $r = -0.98$ ,  $n = 4$ ,  $P = 0.02$ ). Considering all predation events, approximately half of the eggs were consumed *in situ*, and the same proportion of eggs was removed from the stems at all sites. Between one and five egg masses could not be recovered per site, indicating that a maximum of 10 % of the egg masses had been removed entirely from the plant stems.

The average level of predation of eggs on stems surrounded by water was significantly lower than for stems on land with 20 % versus 67 %, respectively ( $Z = 2.57$ ,  $P = 0.01$ ).

### Predation on beetle larvae and pupae

Predation on larvae was highest at Iona South marsh, where, on average, 46 % of all larvae disappeared (Table 3-2), and lowest at Jericho with a disappearance of 22 %. Predation was similar and intermediate at Iona North and Westham. Levels of predation on the larvae were not related to plant damage levels ( $r = 0.71$ ,  $n = 4$ ,  $P = 0.29$ ).

Predation on pupae was lowest at Iona North marsh where, on average, 11 % of all pupae disappeared. The predation rates of pupae were intermediate at Iona South marsh and Jericho, respectively. Interestingly, pupal predation rate was highest at Westham: on average, only 55 % of the pupae were retrieved. Droppings at this site indicated the presence of a high abundance of rodents. Pupal recovery rates were not significantly related to plant defoliation levels in 2001 ( $r = -0.5$ ,  $n = 4$ ,  $P = 0.5$ ).

#### Densities of crawling predators and levels of predation among sites

The density of the predatory beetles and spiders was similar at all sites, but the density of ants varied (Fig. 3-4). At Iona South marsh, the mean density of ants was at least 20 times higher than at any other site. The only ant species found in May and June at this site was *Myrmica lobifrons* Pergande.

There was no correlation or consistent trend between the density of any of the crawling invertebrate groups in the pitfall traps in May, when egg predation was measured, and the observed egg predation rates (ants  $r = 0.72$ ,  $P = 0.28$ ; beetles  $r = -0.61$ ,  $P = 0.40$ ; spiders  $r = -0.37$ ,  $P = 0.62$ ). Larval mortality from predation in June was correlated with the density of spiders ( $r = 0.95$ ,  $P = 0.05$ ), but not ants ( $r = 0.83$ ,  $P = 0.17$ ) or beetles ( $r = -0.4$ ,  $P = 0.6$ ) in the traps. The number of lost pupae did not vary with the abundance of ground-dwelling beetles ( $r = 0.74$ ,  $P = 0.26$ ), spiders ( $r = 0.71$ ,  $P = 0.29$ ), or ants ( $r = 0.43$ ,  $P = 0.57$ ) in the pitfall traps in July.

## Plant quality

Plant quality, measured as the weight and as the survival of larvae on caged plants, was not significantly associated with the mean levels of damage to plants among sites (larval weight:  $r = -0.56$ ,  $n = 4$ ,  $P = 0.44$ ; larval survival:  $r = -0.07$ ,  $n = 4$ ,  $P = 0.93$ ), but the average larval weights and survival levels differed significantly between sites (larval weight:  $F_{3, 36} = 4.1$ ,  $P = 0.01$ ; larval survival:  $F_{3, 36} = 5.78$ ,  $P < 0.01$ ; Table 3-2). Larval weight was lowest at Westham, where a larva weighed, on average, only 55 % of the average larval weight at Iona North.

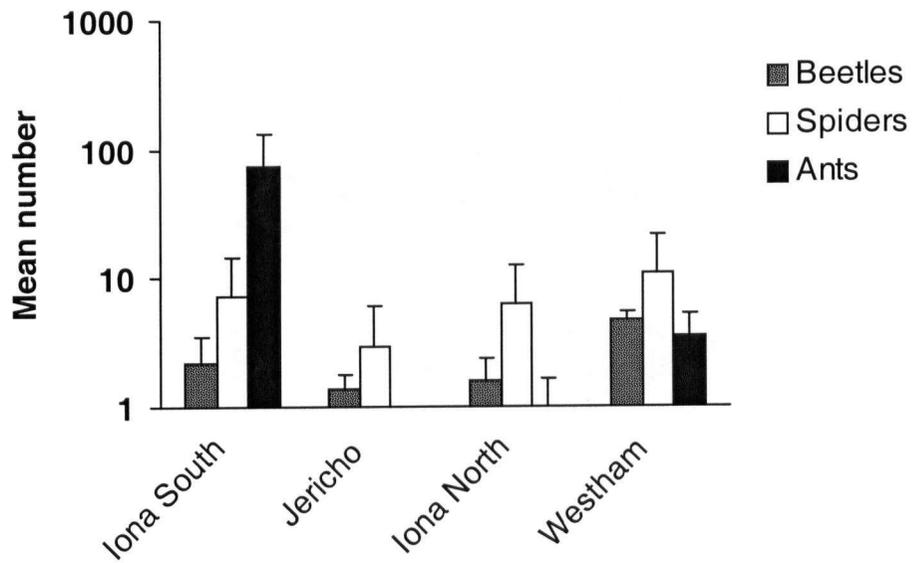
Larval survival was significantly lower at Jericho Park when compared to the other sites (Tukey-Kramer HSD test; Table 3-2), and many of the experimental plants at this site were withered apparently due to a fungal infection.

## *Beetle feeding damage and plant performance*

### *Lythrum* performance at sites without beetles

As suggested earlier, the correlation analysis of beetle damage and impact on plants is compromised by the absence of plant measurements before beetle attack, but variation in *Lythrum* performance at three tidal sites lacking beetles serves to indicate among-site variation due to factors other than beetle feeding.

Flower bud density (Table 3-3) was the only characteristic that did not vary among tidal sites (Welch ANOVA,  $F_{2, 23.3} = 0.21$ ,  $P = 0.81$ ).



**Fig. 3-4** Mean number (+ SEM) of predatory beetles, spiders and ants caught in five pitfall traps at four experimental sites in May of 2001.

**Table 3-3** Measures (mean  $\pm$  SEM) of *Lythrum* reproductive and vegetative performance at three tidal sites, MacDonald, Deas and Ladner. Variables defined as follows: Length Infl. – the average length of the terminal inflorescences of the five tallest stems per quadrat in 2000; Flower buds – the average number of flower buds in 5 cm of the terminal inflorescences of the five tallest stems per quadrat in 2000; Number Infl. – the average number of inflorescences of the five tallest stems per quadrat in 2000; Flower index – the computed average number of flowers per quadrat in 2000; Stem Height – the average stem height of the five tallest stems per quadrat in 2000; Dry Biomass – the computed average dry biomass per quadrat in 2000; Stem Density Ch. – change in stem density from 1999 to 2002 in percent; Dry Biomass Ch. – change in dry biomass from 1999 to 2002 in percent. *P* indicates the probability that all means are equal; nr = not recorded.

Variable	MacDonald	Deas	Ladner	<i>P</i>
Length Infl. (cm)	17.9 $\pm$ 2.6	34.8 $\pm$ 1.3	20.1 $\pm$ 1.3	<0.001
Flower Buds	50.8 $\pm$ 3.7	49.5 $\pm$ 1.8	53.3 $\pm$ 4.2	0.81
Number Inflor.	2.7 $\pm$ 0.5	1.5 $\pm$ 0.1	1.4 $\pm$ 0.1	0.04
Flower Index	6216 $\pm$ 1566	11155 $\pm$ 998	3487 $\pm$ 630	<0.001
Stem Height (cm)	83.5 $\pm$ 3.8	111.5 $\pm$ 3.8	90.2 $\pm$ 3.3	<0.001
Dry Biomass (g)	nr	431.4	124.6	
Stem Density Ch.	+ 57	- 12	+ 4	
Dry Biomass Ch.	+ 71	- 20	+ 12	

The length of the terminal inflorescence (Welch ANOVA,  $F_{2, 23.0} = 38.9$ ,  $P < 0.001$ ), the number of inflorescences per stem (Kruskal-Wallis test,  $\chi^2 = 6.3$ ,  $df = 2$ ,  $P = 0.043$ ), the flower index (Welch ANOVA,  $F_{2, 23.7} = 24.6$ ,  $P < 0.001$ ) and the height of the tallest stems ( $F_{2, 42} = 15.25$ ,  $P < 0.001$ ), all varied significantly among sites without beetle feeding. Moreover, considering all eight plant characteristics recorded, *Lythrum* individuals at sites without beetle attack (Table 3-3) were not generally more vigorous than attacked plants (Table 3-4). In fact, plants with the highest average value recorded for five of the characteristics examined were found at sites with beetles. Thus, the following comparisons should but be interpreted with caution.

#### *Lythrum* performance at sites with beetles

Larval densities (Table 3-4) were strongly correlated with *Lythrum* defoliation levels ( $r_s = 0.94$ ,  $n = 6$ ,  $P = 0.005$ ). Larval feeding apparently had a negative impact on the reproductive performance of *Lythrum* plants (Table 3-4): both the length of *Lythrum* inflorescences and the flower bud densities of the inflorescences were significantly negatively correlated with the observed feeding damage (both  $r_s = -0.93$ ,  $n = 6$ ,  $P = 0.008$ ). The flower index, a measure of reproductive output per stem, dropped significantly with increasing feeding damage ( $r_s = -0.99$ ,  $n = 6$ ,  $P < 0.001$ ), and seed set was completely suppressed when the herbivore damage was at least 60 %. Insect feeding damage and the number of inflorescences per stem were not related ( $r_s = -0.1$ ,  $n = 6$ ,  $P = 0.87$ ).

*Lythrum* abundance, measured as dry biomass per square meter, was inversely related to feeding damage ( $r_s = -0.89$ ,  $n = 6$ ,  $P = 0.019$ , Table 3-4). This seemed to be

**Table 3-4** Average  $\pm$  SEM *Galerucella* density and measures of *Lythrum* performance in 1 m<sup>2</sup> quadrats at each of six non-tidal sites. Stem Density Ch. and Dry Biomass Ch. are changes from 1999 to 2002 in percent of 1999. \*See Table 3-3 for list of abbreviations; see Methods for details.

Variables*	Boundary Bay	Chilliwack	Colony Farm	Iona South	Jericho	Westham
Larval Density	9.5 $\pm$ 1.2	44.5 $\pm$ 6.9	4.6 $\pm$ 1.1	14.3 $\pm$ 3.0	21.4 $\pm$ 3.5	18.1 $\pm$ 6.4
Defoliation (%)	7.3 $\pm$ 1.6	88.0 $\pm$ 0.0	0.6 $\pm$ 0.4	4.8 $\pm$ 1.5	59.4 $\pm$ 6.5	13.1 $\pm$ 2.4
Length Inflor. (cm)	5.8 $\pm$ 1.8	0.0	39.7 $\pm$ 3.6	14.2 $\pm$ 3.2	0.0 $\pm$ 0	7.6 $\pm$ 2.3
Flower Buds	28.4 $\pm$ 8.7	0.0	68.9 $\pm$ 2.3	26.4 $\pm$ 6.1	0.0 $\pm$ 0	18.6 $\pm$ 4.5
Number Inflor.	6.7 $\pm$ 1.4	0.0	4.4 $\pm$ 0.5	5.4 $\pm$ 0.9	0.0	6.3 $\pm$ 0.9
Flower Index	3810	0	59729	5635	0	1386
Stem Height (cm)	93.6 $\pm$ 2.8	87.4 $\pm$ 3.6	105.2 $\pm$ 5.5	110.8 $\pm$ 5.0	52.4 $\pm$ 4.2	85.5 $\pm$ 5.4
Dry Biomass (g)	151.4	32.5	399.0	133.4	5.5	97.8
Stem Density Ch.	+ 77	- 52	+ 136	+ 42	- 33	+ 26
Dry Biomass Ch.	+ 41	- 83	- 51	+ 224	- 73	- 99

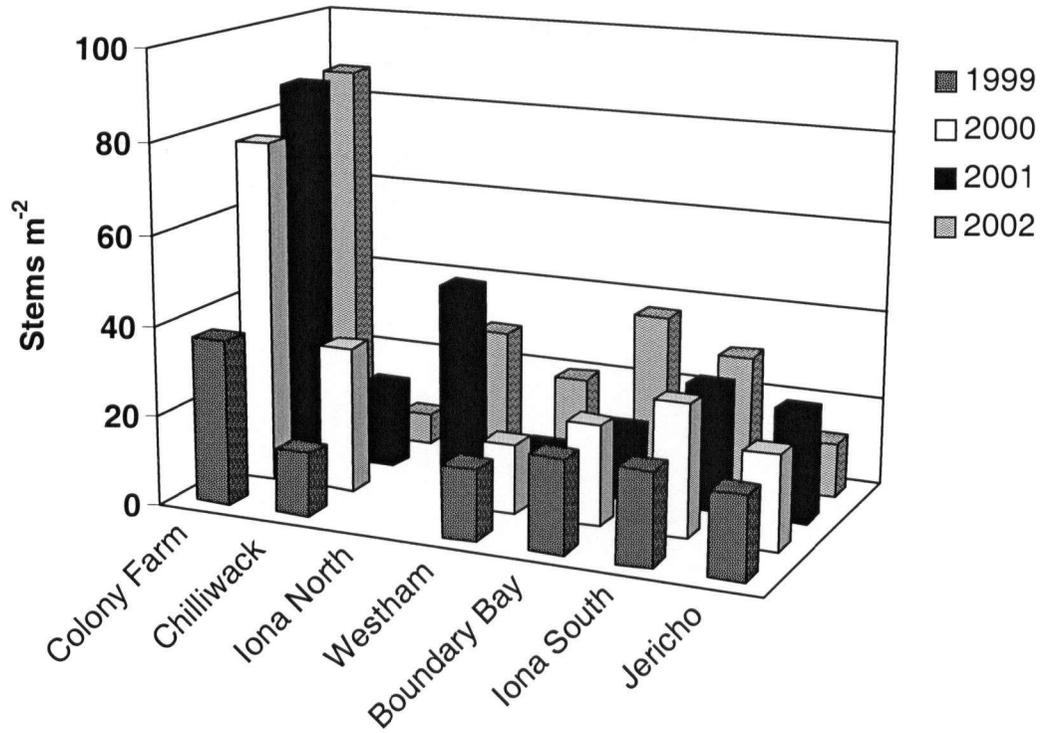
mostly due to reduced size of the individual stems ( $r_s = -0.77$ ,  $n = 6$ ,  $P = 0.072$ ), rather than a change in stem density ( $r_s = -0.37$ ,  $n = 6$ ,  $P = 0.47$ ).

#### Beetle feeding damage and *Lythrum* abundance

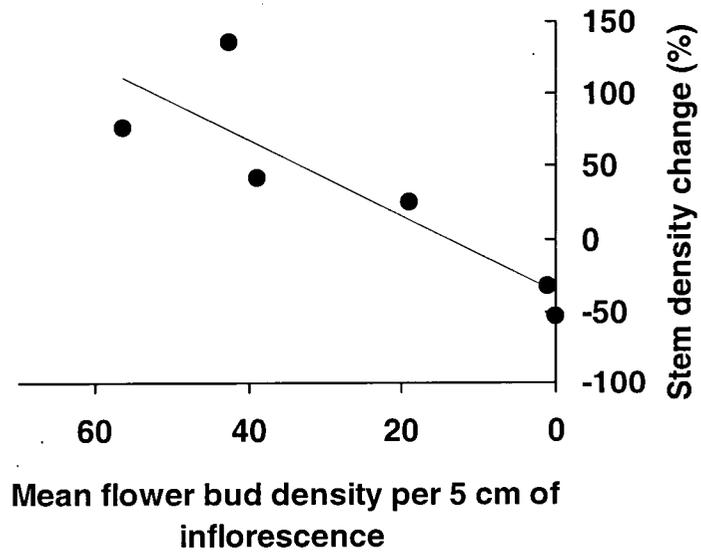
*Lythrum* stem density showed considerable spatial and temporal variation (Fig. 3-5, Appendix 1), ranging from 7 (Chilliwack in 2002) to 87 stems per square meter (Colony Farm in 2002). Between 1999 and 2002, stem density only decreased significantly at one site, Chilliwack, where *Lythrum* plants had experienced complete defoliation for at least four consecutive years (Mann-Whitney U test,  $Z = -2.296$ ,  $P = 0.021$ ). At the Jericho site, stem density in 2002 was 55 % lower than in 1997 ( $Z = 2.4$ ,  $P = 0.016$ ), when the site was first monitored, but unchanged since 1999 ( $Z = -1.44$ ,  $P = 0.15$ ).

In contrast to my prediction, herbivory was related to plant density changes. Considering all non-tidal sites, there was a significant and positive association between the average feeding damage between 1999 and 2002, measured by the mean flower bud density, and the relative changes in stem density over this period ( $r_s = 0.94$ ,  $n = 6$ ,  $P < 0.005$ ). Stem densities only decreased when the feeding activity was so intense as to inhibit flowering for four years, otherwise they increased; interestingly, however, plant stem density increased less when the average feeding damage was more intense (Fig. 3-6). Dry biomass changes were not associated with average flower bud densities during these four years ( $r_s = 0.66$ ,  $n = 6$ ,  $P = 0.16$ , Table 3-4).

At one tidal site, stem density changes also occurred although no control agents were present. At MacDonald Beach Park, *Lythrum* stem density increased significantly from



**Fig. 3-5** Mean number of *Lythrum* stems per m<sup>2</sup> at the monitoring sites from 1999 to 2002. Data for Iona North not recorded in 1999 and 2000. Tidal areas not shown. Error bars omitted for clarity. See Appendix 1 for data.



**Fig. 3-6** Relationship between the average beetle feeding damage, measured by the mean flower bud density per 5 cm of inflorescence of *Lythrum* from 1999 to 2002, and changes in stem densities (in percent of stem density in 1999) between 1999 and 2002 at non-tidal sites ( $n = 6$ ). Note the reverse order of flower bud density on the x-axis to reflect increasing beetle feeding damage. The correlation is significant ( $r_s = 0.94$ ,  $P < 0.01$ ).

an average of  $32.2 \pm 5.7$  to  $50.7 \pm 5.4$  stems (Table 3-3; Mann-Whitney U test,  $\chi^2 = 6.2$ ,  $P = 0.01$ ). However, at Ladner marsh (1999:  $19.3 \pm 2.6$  stems; 2002:  $20.1 \pm 1.8$  stems) and Deas (1999:  $54.7 \pm 4.9$  stems; 2002:  $48.1 \pm 3.2$  stems), there were no significant changes (Ladner marsh: Welsh t-test:  $t_{1, 23.7} = 0.25$ ,  $P = 0.80$ ; Deas: Welsh t-test:  $t_{1, 15.4} = 1.12$ ,  $P = 0.27$ ).

#### **4. Discussion**

The biological control of *Lythrum* by the beetle *Galerucella californiensis* is generally considered to be successful (Julien and Griffiths 1998, Landis et al. 2003, Lindgren 2003), but may only be partially so in coastal British Columbia. The success of a biological control project is dependent on both the density of control agent and the control agent's ability to generate the feeding damage that reduces the weed's biomass and density. My results (Table 3-5) indicate that the beetle can be successful at reducing *Lythrum* stem density in non-tidal areas, although the presence of general predators may delay or hinder beetle outbreaks in certain areas. In tidal areas, however, the beetles are not successful due to their inability to establish.

##### *Variability in beetle feeding damage*

Four years of monitoring revealed high spatial and temporal variability in beetle feeding damage among sites. This variability is not likely to be due to differences in the

**Table 3-5** Summary of experiments to determine the effectiveness of *Galerucella californiensis* as a control agent of *Lythrum salicaria*.

Experiment	Result
Monitoring	Variation in beetle feeding damage among sites and over time. Beetles cannot persist in tidal areas.
Beetle survival in tidal areas	Beetle larvae get washed away and cannot pupate successfully.
The roles of predation and plant quality on beetle feeding damage in non-tidal areas	Predation on beetle eggs, but not on larvae or pupae, or plant quality, is related to beetle feeding levels among sites.
The influence of standing water on egg predation levels	Eggs on <i>Lythrum</i> stems in standing water suffer significantly less predation than eggs on stems on land.
Density of crawling predators and predation levels	The predation on larvae was related to spider density, but the predation levels on eggs and pupae were not related to the density of ants, predatory beetles or spiders among sites.
Impact of beetle feeding on <i>Lythrum</i> performance	Defoliation levels were related to measures of <i>Lythrum</i> vegetative and reproductive performance. Flower bud density is a sensitive measure of feeding damage.
Impact of beetle feeding on <i>Lythrum</i> abundance	<i>Lythrum</i> stem density only declined at one site following four years of intense feeding. The average feeding damage over the four-year monitoring period was inversely related to <i>Lythrum</i> stem density.

time during which the beetles were present at the sites, since most releases occurred in 1997.

The observation that, at some sites, high defoliation levels occurred in 1999, two years after the releases, indicates that the beetle can show fast population growth. The increase in the number of sites at which high levels of defoliation occur shows that the beetles are not generally prevented from reaching outbreak densities by predators, parasitoids or local environmental conditions. It remains to be seen whether the non-tidal sites, at which no beetle outbreaks were observed during the monitoring period, will experience higher levels of beetle feeding in the future. However, the apparent inability of the beetles to establish at tidal sites suggests that *G. californiensis* is not a suitable control agent in these disturbed conditions.

#### *Beetle survival in tidal areas*

Consistent with the hypothesis that disturbances affect beetle survival and establishment, I found that the tidal water movement displaced *G. californiensis* larvae over the short term. Repeated disturbance events could severely impact larval survival, especially in early June, when *Lythrum* plants are small and completely submerged during high tide. Neonate larvae may be especially vulnerable due to their presumably reduced ability to hold on to the plant surface. Given the high rate of disappearance of larvae, I suggest that tidal disturbances may explain the failure of beetles to establish at any of the tidal sites.

While I do not know whether disturbance, predation or both account for the disappearance of egg masses at the tidal sites, the overall disappearance of eggs at tidal sites was not higher than at a non-tidal site. Neither was there evidence for

reduced viability of the beetle eggs at tidal sites, as indicated by the presence of neonate larvae. However, prolonged exposure to tidal conditions could impair egg viability and would not have been detected, since the observed larvae experienced a maximum of two weeks of tidal conditions.

My results also show that *G. californiensis* beetles could not successfully pupate when the soil was consistently flooded. The conditions in the laboratory experiment reflect conditions found in some areas (Ladner Marsh, Jericho Park) where the water table was consistently high due to poor drainage or high precipitation in some years. Under flooding conditions, beetles may pupate in the aerenchyma (spongy stem tissue with air passages) of *Lythrum* (Schooler 1998), a tissue which is only formed in standing water (Lempe et al. 2001). However, aerenchyma tissue was not observed in *Lythrum* stems in two of the tidal wetlands, presumably because good drainage conditions allowed for sufficient gas exchange at low tide.

The failure of biological control in tidal areas raises concerns about the impact of *Lythrum* on the native plant communities and the potential of these areas to serve as a consistent seed source from which new areas can be infested. The question of how *Lythrum* is regulated in tidal areas in its native range provides a valuable opportunity for ecological study and could also help develop effective management strategies for these habitats.

The role of refuges has received a lot of attention in the theory and practice of biological control and ecology in general (e.g. Murdoch et al. 1989, Begon et al. 1995, Milchunas and Noy-Meir 2002). Theory predicts that prey refuges may stabilize predator-prey interactions and play an important role in the persistence of the predator-prey system (Begon et al. 1995). If this hypothesis is applicable in plant-herbivore systems, the existence of refuges could be favorable to the persistence of the beetle-

*Lythrum* system, because the invulnerable plants in the refuge provide a steady supply of seeds, thereby reducing the risk of beetle extinction once *Lythrum* is eliminated as Lindgren (2003) observed at his study site. Clearly, the benefits and risks of the *Lythrum* infestations in tidal areas have still to be elucidated and weighed against each other.

#### *Variation in predation, predators and plant quality among sites*

Among the factors studied here, only egg predation was significantly associated with the levels of herbivore feeding damage. Thus, my results suggest that egg predation, but not food plant quality, affect beetle densities across sites. This is consistent with a review by Walker and Jones (2001), which concluded that predation, not resources, predominantly accounts for the mortality of immature insect herbivores. Egg predation has been previously suggested as a factor potentially affecting *G. californiensis*' success as a biological control agent (Nechols et al. 1996, Wiebe and Obrycki 2002). However, this study is the first to provide evidence for a link between predation in *G. californiensis* and biological control success by using data collected under field conditions. Although I cannot differentiate between the causes of varying beetle densities and their consequences, my study suggests that predation could be an important factor in determining beetle density and defoliation levels. Thus, I do not reject the predation hypothesis.

While predation on larvae has been suggested as a force that could impact beetle populations (Sebolt and Landis 2002), I did not find evidence for a consistent role of larval predation in my study. Similarly, I did not find a general role for plant quality in influencing beetle populations, although there are observations that suggest that plant quality varied among sites and may play a role in certain life-history stages. First, egg

mass density in relation to adult density varied across the four experimental sites, and this suggests that plant quality may influence oviposition behavior (Walker and Jones 2001). Second, larval weights and larval survival differed between sites suggesting that plant quality affects larval growth. However, there is no consistent pattern across developmental stages indicating superior plant quality at any site: while the larval weight on enclosed plants was highest at Iona North marsh, larval survival was not, and the ratio of egg masses to adults was lowest at this site when compared to the other three sites. Thus, if plant quality indeed plays a role in influencing beetle density in this system, the effects it has in one life-history stage may be counteracted by the effect in another.

Plant quality and predation could be related if reduced plant quality slows larval growth and thus increases exposure to predators (Lawton and McNeill 1979). However, I did not find evidence for this: the weight of protected larvae was lowest at the site with complete plant defoliation, Westham, when compared to the other three sites at which larval weight was measured.

It is also possible that I did not find significant effects of plant quality on beetle survival because I used second and third instar larvae to estimate levels of predation, while younger larvae maybe more susceptible to predators (Sebolt and Landis 2002).

#### *Influence of water level on egg predation*

Eggs on plants surrounded by standing water had higher survival than accessible eggs. This indicates that predators are predominantly crawling, not flying, and cannot access eggs protected by the surrounding water. Thus, *Lythrum* plants in standing water appear to act as a predator refuge for *G. californiensis*.

If this finding applies generally, however, I predict that beetle outbreaks should start more commonly in years when winter precipitation is plentiful and in patches of plants in standing water, where high water levels in the spring protect the eggs. A significant decrease of the water level by the end of June observed at most sites would allow the beetles to pupate successfully in the soil. Casual observations are consistent with this prediction. At the Iona sites I found that plants in standing water in early spring were more likely to be defoliated. At Iona North marsh, the first, completely defoliated stems were observed in a small patch of plants in a swale that was flooded until mid-summer in 1999. These plants were defoliated in every subsequent year, irrespective of the average defoliation of plants in the surrounding area. At the Iona South marsh, individual plants near the water's edge, which had been surrounded by standing water in spring, were also defoliated in July of 2001, but not plants on dry land. Thus, at least at sites where *Lythrum* grows in standing water, refuges from predation may allow the herbivores to escape to high densities, resulting in small-scale (individual plants) or large-scale (entire area) defoliations.

#### *Densities of crawling predators and levels of predation*

Of the ground-dwelling predators, spiders appeared to contribute to the predation of larvae in June, as indicated by a significant correlation between their density and larval predation. However, I did not find evidence for a relationship between any of the invertebrate groups present in the pitfall traps in May and the predation of *G. californiensis* eggs. A possible explanation is that some predator groups are not accurately reflected in the pitfall traps, such as predators that reside predominantly on the plants. For example, Blossey (1991) observed mites feeding on *Galerucella* spp.

eggs early in the growing season and when egg density was high, but mites were rarely present in my samples. Moreover, invertebrate predators in the pitfall traps were only assigned to three large taxonomic groups. If predation was mainly due to one or few species, the taxonomic resolution of the pitfall trap samples may have been insufficient to detect a relationship between predation levels and these species.

Ant abundance at the Iona South site, the site with the least beetle feeding damage, was considerably higher than at the other sites. While the abundance of a particular predator does not always imply that it feeds on a particular target species, numerous studies (e.g. Leslie 1982, Campbell et al. 1983, Youngs 1983) suggest that ants can play an important role in herbivore reduction. Ballard and Mayo (1979) report that, in a laboratory experiment, ants of the species *Myrmica americana* Weber removed Chrysomelid eggs on the soil surface and, in the field, frequented the base of the host plant. Crawley (1989b) emphasizes the general vulnerability of insect eggs to ant predation and its significance for the failure of biological control.

The larval densities of *G. californiensis* at Iona South marsh were much lower than expected from adult densities when compared to the other sites. This suggests that high mortality occurred during the egg or larval stage. The effective regulation of the herbivores there was possibly due to the high abundance of predatory ants. The observation that no *G. californiensis* outbreak occurred at Iona South marsh since the beetle was released, while insect outbreaks occurred at the other three experimental sites at least once between 1999 and 2002 (Fig. 3-2), further supports the interpretation that local predators may successfully prevent beetle outbreaks at this site.

Since the densities of all predator groups were similar among three experimental sites, but egg predation varied, factors other than predator abundance could account for

the different egg predation levels. These factors may include predator satiation, if predation rate decreases when herbivore abundance increases.

Predator satiation has been commonly suggested to occur in insect outbreak situations (e.g. Roland 1994, Crawley and Long 1995, Harrison and Wilcox 1995, Tanhuanpää et al. 1999), and could account for the inability of the predators to regulate their prey. When predator satiation occurs, predation levels, not predator abundance, are lower at the outbreak sites. Although predator satiation is a possible explanation for the difference in predation level between the study sites with intermediate and complete defoliation, but similar predator abundance, I did not have supporting evidence such as inverse density-dependence in beetle survival rates (Harrison and Wilcox 1995). Further experimental work is needed to determine whether predator satiation is operating.

#### *Impact of beetle feeding on individual host plant performance*

I infer from my results that the beetles are effective at reducing seed output and biomass of *Lythrum* plants in the short term. Although the interpretation of these correlational data must be limited, the following evidence supports my conclusion: first, flower bud density did not vary among sites without control agents. If this is also true for non-tidal sites, the strong association of beetle feeding activity and flower bud density supports the interpretation that the beetles caused this reduction in reproductive performance. Second, my findings are in agreement with other studies (Blossey and Schat 1997, Katovich et al. 2001, Lindgren 2003) and confirm that even moderate herbivore damage has a negative effect on *Lythrum* seed production. Thus, given the strong association between beetle feeding damage and both *Lythrum* reproductive and vegetative performance in the short term, I do not reject the hypothesis that beetles

reduce plant performance in the short term. My results also concur with findings in goldenrod plants, *Solidago altissima* L., where plants respond to feeding by native herbivores with a reduced seed production, but conserve the resource allocation to established plant individuals (Root 1996).

At least in the short term the reduction in seeds is not likely to translate into changes in the plant's population dynamics, given the plants' longevity and large seed bank in the soil (Welling and Becker 1990). It is possible that even substantial reductions in seed output may not translate in plant density changes, as has been observed in a number of species (see review in Myers and Risley 2000), among them knapweed, *Centaurea diffusa* Lam., where an estimated reduction in seed output of 95 % did not result in a reduction in plant density. Nevertheless, the potential of *Lythrum* to spread is reduced because propagation and spread occur generally by means of seeds (Mal et al. 1992).

Some of the variation in plant performance is likely due to environmental conditions, as indicated by the variation at sites without the beetle, and this likely reduces the power to detect effects of beetle feeding on plant performance. For instance, I found only a marginal effect of beetle feeding on stem height, although significant effects have been reported in other studies (e.g. Lindgren 2003). Moreover, measuring only the performance of the tallest stems, which are likely grown by mature plants, may result in a conservative estimate of beetle feeding impact, since the ability to compensate for feeding damage is likely to be greater in mature plants than in seedlings and young plants (Blossey and Skinner 2000).

## *Impact of beetle feeding on Lythrum abundance*

Previous research has shown that *Lythrum* is able to tolerate intense feeding of *Galerucella* spp. for more than two years before mortality occurs (Katovich et al. 1999, Blossey and Skinner 2000). Consistent with this result, I found that *Lythrum* stem density decreased only at the Chilliwack site, which experienced the most consistent and intense feeding of all sites over the four-year monitoring period. *Lythrum* plants contain extensive carbohydrate reserves below- and above-ground (Mal et al. 1992, Katovich et al. 1999) that allow the plants to grow new shoots a few weeks after defoliation (personal observation), intense and consistent defoliation, as observed at the Chilliwack site, likely depletes the reserves, eventually resulting in plant mortality. Thus, my findings support the hypothesis that prolonged herbivore attack is required to reduce plant density and that four years of intense feeding are apparently sufficient to cause plant mortality.

To my knowledge this is the first study to document that cumulative feeding effects, even if small to moderate, are associated with stem density changes in *Lythrum*. Although only intense and consistent grazing appears to result in a decline of *Lythrum* density, plant density increased less when the average feeding damage was more intense. This result suggests that consistent feeding for a period of four years, irrespective of its intensity, affects the plant population by potentially slowing its growth. Thus, my results are not consistent with the hypothesis that only intense feeding has an impact on *Lythrum* abundance.

One or a combination of several of the following reasons may have prevented detecting a significant reduction in stem density at more sites. First, yearly fluctuations in stem densities due to environmental variation may mask trends in stem densities. For

example, variation in *Lythrum* stem density was considerable in tidal areas, where the control agent did not establish and were likely due to changing environmental conditions. The finding that *Lythrum* stem density at the Jericho site apparently declined from 1997 to 1999 could also be due to yearly fluctuations in *Lythrum* stem density.

Second, increased light levels at ground level due to *Lythrum* defoliation allow populations to draw on their abundant seed banks (Welling and Becker 1990) by encouraging seedling growth and survival since the timing of defoliation in June coincides with seedling emergence (Mal et al. 1992). Thus, a decrease in *Lythrum* stem density may be delayed due to increased seedling survival.

Third, in well-established *Lythrum* plants vegetative growth likely compensates for, and masks, the effects of herbivore feeding. The observed increase in stem densities at almost all sites is consistent with the observation that established plants increase their lateral size and stem numbers annually (Mal et al. 1992), resulting in individuals with more than 130 ramets (Anderson 1991). In contrast, young plants may not be able to compensate to the same extent (Hendrix 1988). Consequently, the density of established plant may not decrease, but plant population growth could be reduced. Thus, if herbivory is persistent, but not intense, it may reduce, but not stop or reverse, the rate of *Lythrum* population growth.

Fourth, if control agent feeding is not intense, it may reduce the biomass of individual *Lythrum* stems, but not their number, and measuring stem density changes would not accurately reflect the impact of herbivory.

The following limitations highlight the need for a manipulative study to confirm the result of my analysis. First, my conclusions are based on correlational analyses and caution must be used in interpreting the results. The fact that I also found a significant change in the *Lythrum* stem abundance in one tidal site, MacDonald, although control

agents were not established there, indicates that at least a portion of the stem density changes attributed to beetle feeding is due to yearly fluctuations and sampling error.

Second, I made the simplistic assumption that there is no upper limit to the stem density, since I projected a growth in stem density proportional to the stems present in 1999. This is clearly not a generally valid assumption, since intraspecific competition will slow *Lythrum* stem growth when resources become scarce. At most sites, *Lythrum* is not evenly distributed, but occurs in dense clumps. In these situations, sampling is carried out at the edge of the infestation, where growth conditions are likely more suitable for seedlings. Thus, my finding that there is a cumulative feeding effect may only apply to the specific conditions at the edge of an infestation, where seedlings and young plants occur more frequently than in the dense clumps. Possibly, the increased mortality among young plants attributed to beetle feeding would also occur without herbivory, but later, when resource competition is more intense.

### *Implications for biological control*

Biological control programs provide a valuable opportunity for ecological research, yielding both insight into ecological processes and applied information to evaluate the biological control program and to guide management decisions.

Two important principles emerge from my study: first, my results highlight the importance of the abiotic environment at the introduction sites for the weed's and the beetle's population dynamics. While interference with native, generalist predators appeared to influence the abundance of a biocontrol agent at a local scale, the abiotic environment may ultimately determine the balance of the trophic interactions and thus, the success of this biological control project at a regional scale. Biological control was

apparently facilitated by the creation of temporary refuges from predation for the biocontrol agent in undisturbed areas when water levels were high in the spring. In contrast, tidal areas act as a refuge for the weed, since disturbances associated with the water movement appear to prevent control agent establishment.

Second, the type of damage inflicted by the biological control agent is affecting a critical stage in the life history of the weed, and can result in a reduction of *Lythrum* abundance in the long term.

Clearly, further research would benefit our understanding of how the dynamics of *Lythrum* and the biocontrol agent are influenced by the existence of plant and herbivore refuges. Further experimental work is also needed to confirm the long-term effects of low levels of herbivory suggested by the monitoring results. This knowledge could guide future management decisions such as the introduction of additional control agents into tidal areas for effective management of *Lythrum* on a regional scale.

## CHAPTER FOUR

### CONCLUSIONS

#### *Risks and benefits of classical biological control*

The problems created by exotic species worldwide highlight the need for sustainable management methods that take into account the sensitivity of the invaded areas. With many alien invaders, biological control is a last resort because there are no viable alternative management methods (Fowler et al. 2000). However, introductions of control agents can potentially have detrimental effects on non-target species and may affect native food webs (Cory and Myers 2000). Thus, the risks and benefits of introductions of control agents for the purpose of a biological control program need to be weighed carefully (McFadyen 1998).

Despite the careful evaluation of the specificity of control agent candidates, precise knowledge of the risk involved in the introduction of a control agent is impossible prior to the introduction; complex interactions with native species can never be realistically simulated. However, it is clear that the risk increases with the number of introduced control agents, and therefore, introductions should be minimized. Unnecessary control agents should not be introduced (Denoth et al. 2002).

In contrast to the evaluation of the risks of biological control, the benefits of control agent introductions can be assessed before control agents are introduced by

measuring the response of native species to an experimental reduction of the target pest.

The aims of this study were twofold:

- 1) To determine the potential benefits of successful biocontrol, I assessed the impact of the European invader *Lythrum salicaria* L. on the rare and endemic wetland species *Sidalcea hendersonii* S. Wats. in British Columbia.
  
- 2) To evaluate the potential of *Galerucella californiensis* as a successful biocontrol agent of *Lythrum* in southwestern British Columbia, I measured the effect of the control agent *Galerucella californiensis* L. on *Lythrum*, and examined factors associated with the varying levels of beetle feeding damage among sites.

### *Conclusions of this thesis*

#### *Impact of Lythrum salicaria on Sidalcea hendersonii*

The results of field surveys and competition experiments do not support the hypothesis that *Lythrum* has a strong impact on *Sidalcea*. Two factors may account for this result. First, the early emergence and tall growth of *Sidalcea* may confer a significant advantage to the plant with regard to its interactions with competitors. Second, due to the environmental conditions and competitive interactions in the marsh, the abundance of *Lythrum* in the marsh does not appear to be so high as to have a strong, negative effect on *Sidalcea*.

The abundance of *Lythrum* in the marsh where it co-occurs with *Sidalcea* did not increase significantly from 1979 to 1999. This suggests that the invasion process was

largely completed by 1979. Thus, based on the available evidence, control of *Lythrum* in tidal areas does not appear to be necessary to protect *Sidalcea*.

#### Biological control of *Lythrum salicaria*

In evaluating the effect of the control agent on *Lythrum*, I documented the spatial and temporal variability of feeding damage to *Lythrum*. Additionally, I investigated the effects of predation and plant quality on the performance of *Galerucella* and related the performance of the host plant to the beetle feeding damage.

The results indicate that the effectiveness of control agent is dependent on site conditions. In tidal areas, the beetles are unable to persist due to high mortality associated with the tidal disturbances. In non-tidal areas, only predation on *G. californiensis* eggs, but not predation on any other life-history stage, or plant quality, was related to beetle feeding damage. The densities of crawling, invertebrate predators were not generally related to predation levels, suggesting that predation did not vary in response to ground predator abundance. Possibly, predator attack levels varied with control agent density, or other types of predators were responsible for the observed predation levels.

High water levels in the spring and their effect on the efficiency of crawling, invertebrate predators may be a significant factor in the initiation of beetle outbreaks, at least at some non-tidal sites. At a site where no beetle outbreak occurred during a four-year period, ants were abundant and may have been capable of regulating the control agent.

*Lythrum* dry biomass and seed output were reduced in response to beetle attack. However, stem density declined at only one site after persistent and severe herbivory

over a four-year period. At most other non-tidal sites, plant stem density increased, and stem density changes were inversely related to the average feeding damage during the previous four years. The control agent may be effective at eventually reducing the density of *Lythrum* at other non-tidal sites if the beetle consistently reaches outbreak densities. However, *Galerucella californiensis* will not be successful at reducing the abundance of *Lythrum* in tidal areas.

*Should other control agents be introduced?*

Continued monitoring in non-tidal areas will show if the density of *Lythrum* declines at more release sites in the future. Presently, the introduction of additional control agents to non-tidal areas in southwestern British Columbia is not advisable, given that *G. californiensis* has shown a capability of reducing the weed, and in consideration of the risk associated with the introductions.

The question as to what control methods are feasible in tidal areas and whether they should be employed cannot be answered soundly without further scientific studies. Careful evaluation should guide future management decisions. The weak impact of *Lythrum* on the rare, endemic *Sidalcea* suggests that employing control methods in tidal areas is not warranted. In addition, the presence of a tidal refuge from beetle herbivory could potentially benefit the biological control by stabilizing the interaction between *Lythrum* and the control agent in non-tidal areas because the invulnerable plants in the refuge provide a steady supply of seeds, thereby reducing the risk of beetle extinction. However, the failure of biological control in tidal areas also raises concerns about the effects of *Lythrum* on wetland species other than *Sidalcea*, and the potential of infested areas to serve as a consistent seed source from which new sites can be invaded.

Since herbicides are banned near water sources in Canada, physical removal of *Lythrum* is the only control method currently available in tidal areas, but its use may be precluded in areas that are sensitive to human disturbance. Knowledge of the factors that account for *Lythrum* control in tidal areas in its native range could potentially provide guidance for selecting complementary control methods in North American wetlands if they are needed.

*How successful is the biological control of Lythrum salicaria?*

McFadyen (1998) advocates the use of the following definitions of the biological success for biological control: 1) Complete success - no other control methods are needed; 2) Substantial success - complementary control methods are still required, but at reduced levels; 3) Negligible success - the management of the weed is still dependent on other control methods. Based on the available results, I conclude that the biological control of *Lythrum salicaria* in southwestern British Columbia is substantially successful. However, it may be still too early to fully recognize the extent of the success of this biological control project, since analyses of success rates should only be done 10-20 years after the last introductions (McFadyen 1998). However, other types of success as defined by Anderson et al. (2000) can be assessed with more certainty.

A number of studies, including this one, have investigated the interactions of the control agent with its new environment and its impact on the target plant, and our improved understanding of the control agent as a consequence of these studies can be interpreted as a scientific success.

The conspicuous inflorescences of *Lythrum* make this plant obvious in infested areas and have frequently prompted an interest of the general public in the plant and its

impact. Interested groups have engaged in the biological control program and successfully reared and released the control agent in various wetlands. The increased awareness of the problem of invasive species and the improved acceptance of biological control with the general public makes this project also a social success.

Legislation at the federal and provincial levels has greatly enhanced weed management efforts for the weed. *Lythrum* has been declared a noxious weed in several Canadian provinces and American States, and the introduction of biological control agents has been approved. The heightened awareness at the political level is also reflected in the release of funding resources needed for weed management. For instance, the City Council of Langley in the Lower Mainland of British Columbia has contributed financial resources to a Naturalist group in the area to rear a control agent that was subsequently released in an infested area. Thus, the increased awareness of public representatives is a major factor in the biological control of *Lythrum* and has contributed to the political success of this project.

## APPENDIX 1

The average number of *Lythrum* stems per m<sup>2</sup> at various sites from 1999 to 2002. Estimates were obtained by sampling 10 to 15 quadrats.

Site	1999	2000	2001	2002
Colony Farm	37	77	87	87
Chilliwack	14	33	19	7
Iona North	nr	nr	44	29
Westham	16	16	9	20
Boundary Bay	21	22	16	37
Iona South	21	30	28	30
Jericho	18	21	25	12
Ladner	19	38	22	20
MacDonald	32	nr	51	51
Deas	55	73	70	48

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