THE IMPACT OF TWO INTRODUCED HERBIVORES ON THE POPULATION ECOLOGY OF *LYTHRUM SALICARIA*: IMPLICATIONS FOR PLANT PERFORMANCE, REPRODUCTION AND COMMUNITY DIVERSITY

Excedera St. Louis

Thesis submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment of the requirements for the M.Sc. degree in the
Ottawa-Carleton Institute of Biology
Department of Biology
Faculty of Science
University of Ottawa

Abstract

The release of biological control agents into the environment is inherently risky: assessment of those risks through on-going, post-release monitoring is very important. Herbivores have the potential to inflict multiple impacts on a host plant's performance and reproduction. Previous research demonstrates that the effects of herbivory on plants include changes to plant architecture, biomass allocation, flowering time and reproductive success, to list a few. Moreover, when herbivory significantly impacts the population ecology of a dominant community member, other species can be indirectly affected, ultimately influencing plant community ecology. Here I describe an investigation into the impacts of two introduced herbivorous biological control agents: the leaf beetle *Galerucella calmariensis* (Coleoptera: Chrysomelidae), and the flower-feeding weevil, *Nanophyes marmoratus* (Coleoptera: Brentidae) on several characteristics of the host plant species, invasive purple loosestrife (*Lythrum salicaria*), and its surrounding community. I collected data on 18 invaded communities from around eastern Ontario, including information on feeding damage and the density of each species of biological control, along with data on purple loosestrife's height and biomass, inflorescence length, inflorescence number and fruit production. The history of each site's colonization by *Galerucella* was also considered. I discovered that the density of both *Galerucella* and *Nanophyes* at a site was negatively associated with *Lythrum* fruit production. However, herbivore density was not significantly associated with *Lythrum* biomass, height or the species richness of the surrounding plant community. This study, conducted 20 years after the initial Ontario release of *Galerucella*, demonstrates that although vegetative traits of *Lythrum* do not appear to be significantly impacted by the presence of *Galerucella* or *Nanophyes*, reproductive traits are. Twenty years is likely too short a time period to adequately assess the impacts of the release on community species richness,

although my data indicate that communities with smaller *Lythrum* plants tend to have higher species richness. This study covered a small geographical area and data collection was conducted for a single season only; adding additional years and/ or sites is recommended.

TABLE OF CONTENTS

List of Tables

List of Figures

Acknowledgements:

I would like to acknowledge my supervisor R. Sargent for her guidance and suggestions. Thank you to other members of our lab, including M. Stastny and J. Russell-Mercier and I. McKechnie for their assistance and support, B. Hall and C. Thomsen and G. Forget for field assistance and to everyone in the lab who made it a supportive and pleasant work environment. I would also like to thank my other committee members N. Cappuccino and H. Rundle as well as those who were able to give me information on previous related projects, including, but not limited to, J. Corrigan, D. McKenzie, D. Parker. Appreciation goes out to the crew at the Algonquin Park Biological Field Station for always being so accommodating and fun as well as P. Bouchard who reconfirmed the identity of *Nanophyes marmoratus*. In addition, thank you to all the people in my life who support, and are aware of, all the decisions I make, especially DStL, CD, CC, JV.

CHAPTER 1: General Introduction and Overview

Biological Control of Invasive Species

Invasive species are both economically and ecologically detrimental; estimates have put their cost to the North American economy in the range of billions of dollars annually (Mack et al. 2000, Pimentel et al. 2005, Colautti et al. 2006). Once established, the total eradication of an invasive species is unlikely (Crawley 1989, Syrett et al. 2000), but management may be possible. In an attempt to make invasive species management less costly and more self-sustaining, biological control agents have been included in and have even replaced traditional management plans or strategies in many parts of the world (Mason and Gillespie 2013). Classical biological control involves the introduction of a natural enemy or enemies (i.e., predators and herbivores) from the invasive species' native range, and has been applied in North America for decades (Mason and Huber 2002, Pearson and Callaway 2003, Mason and Gillespie 2013), although it involves inherent risks (Howarth 1983, Secord and Kareiva 1996, Cory and Myers 2000, Simberloff and Stiling 1996, Carson et al. 2008, Simberloff 2011). In naively assuming to understand the all of the potential ecological impacts of the introduced biological control agents, there have been several infamous examples of serious failures (e.g., the release of the Indian mongoose on Caribbean Islands to control rats (Pimentel et al. 2005), and the release of the cane toad in Australia to control a sugar cane larval pest (Doody et al. 2009)). However, there are also success stories where biological control has apparently been able to mitigate the impacts of invasive species (see DeBach and Rosen 1991, Mason and Huber 2002, Mason and Gillespie 2013). Indeed, success stories such as the control of tansy-ragwort, diffuse knapweed and Klamath weed by introduced herbivores (McEvoy et al. 1991, Bellows 2001, Jensen et al. 2002, Myers et al. 2009) are not uncommon. That said, the success of these programs is difficult to measure

(Syrett et al. 2000, Headrick and Goeden 2001, Myers et al. 2009). One issue is that it is difficult to determine at what point we should consider an invasive species 'managed'. Related to this, there is uncertainty surrounding the length of time monitoring (and funding for monitoring) of the impacts should be required post-release.

Factors that may contribute to a species' propensity to become invasive have often been described (e.g., Strauss et al. 2006, Lau 2008, van Kleunen et al. 2010, Bennett et al. 2011) along with theories about what drives invasiveness, such as the evolution of increased competitive ability hypothesis (EICA) (Blossey and Nötzold 1995), and the enemy release hypothesis (ERH) (Keane and Crawley 2002). Biological control programs rely on the ideas outlined in these hypotheses. The EICA and the ERH both hinge on the idea that introduced species may experience a competitive advantage in the absence of their natural enemies (i.e., specialist predators, herbivores, parasites, parasitoids). This advantage may enable introduced species to establish and eventually become invasive (Crawley 1986, Thompson et al. 1987, Lawton and Brown 1986, Malecki et al. 1993, Blossey and Nötzold 1995, Williamson 1996, Williamson, M. and A. Fitter. 1996, Keane and Crawley 2002, Wolfe 2002, Müller-Schärer et al. 2004), outcompeting native species for resources such as nutrients, space, light and pollinators (Brown and Mitchell 2001, Bjerknes et al. 2007, Chun et al. 2007, King and Sargent 2012). The goal of classical biological control is to reduce this competitive advantage of an invasive species by reintroducing specialist predators from its native range.

Nationally, biological control agents are regulated by the Canadian Food Inspection Agency, under the Plant Protection Act (1990) and the Pest Control Act (2002), to ensure safety and quality (Mason and Gilllespie 2013). Before their approval, biological control agents are typically assessed by taxonomists, ecologist and experts in biological control for several attributes including climatic compatibility, host specificity, feeding preference as well

as the potential for multi-species interactions, including the displacement of, or inter-breeding with, native species (Blossey et al. 1994a, Blossey et al. 1994b, Ruesink et al. 1995, Lindgren et al. 2002, van Lenteren et al. 2006, Mason and Gillespie 2013). Depending on the agents' feeding and reproductive ecology, combinations of more than one species may, or may not, be advisable (Denoth et al. 2002). Ideally, the characteristics of the target invasive species should also be taken into consideration when deciding if it is a logical candidate for biological control (Paynter et al. 2012). As all organisms coexist and interact with a myriad of other species in inherently complex ecosystems, potential interactions between the biological control agents and other species should be taken into consideration (Miller and Travis 1996, Brody 1997, Strauss and Irwin 2004, Poveda et al. 2005, Strauss et al. 2006, Swope and Parker 2010, Jones and Gomulkiewicz 2012, Swope and Parker 2012). However, even if select pair-wise interactions are studied, it is not possible to study or even to foresee all future interactions. In addition, the effects of one organism on a target species may be heightened or reduced due to the presence of, or the interactions with, additional enemies, or even mutualists, in the community (Ehler and Hall 1982, Ferguson and Stiling 1996, Morris et al. 2007, Barber et al. 2012). Furthermore, once in its new environment, evolved changes in either the agent or the invasive may lead to unexpected outcomes. Nevertheless, more than 400 species of invertebrates have been approved for use as biological control agents in Canada, most being entomophagous (i.e., for the control of insect pests) and the remaining 22% being herbivorous (Mason and Gillespie 2013).

Effects of Herbivory

Herbivory has the potential to impact plant fitness both directly and indirectly. For example, herbivores can affect the overall growth and architecture of the host plant (Grange 1990, Karban and Strauss 1993, Schat and Blossey 2005) as well as the allocation of

resources to above- or below-ground growth (root:shoot biomass allocation) (Karban and Strauss 1993, Nötzold et al. 1998, Hunt-Joshi et al. 2004). Root-feeders can limit the amount of nutrients and water reaching the above-ground portion of the plant, resulting in the a variety of responses (Brown and Grange 1989, Brown and Grange 1990, Müller-Schärer and Brown 1995, Hunt-Joshi et al. 2004), including increased susceptibility to additional herbivores and pathogens (Master et al. 2001). Sap-suckers can induce similar results (Vranjic and Gullan 1990, Hambäck 2001). Foliage–feeders reduce plant performance by altering growth rates, biomass allocation and photosynthetic rate as leaf material is grazed (Grange 1990, Zangerl et al. 2002, Hunt -Joshi et al. 2004). Herbivory can also affect a plant's reproductive biology (Quesada et al. 1995, Mutikainen and Delph 1996, Lehtilä and Strauss 1997, Poveda et al. 2003, Pellegrino and Musacchio 2006, Hladun et al. 2009), pollination rates (Agren 1996, Lehtilä and Strauss 1999, Strauss et al. 2001), lifespan and/ or likelihood of survival (Rausher and Feeny 1980, Strauss 1991, Doak 1992, Brody 1997).

Herbivores can also directly influence plant fitness by feeding on the reproductive structures themselves. For example, flower-bud grazers may delay or prevent the plant from reaching the reproductive stage (Blossey and Schat 1997, Mason and Huber 2001), and flower- or seed-feeders may directly reduce seed or fruit set by ingesting reproductive structures (Meyer 1993, Brody 1997, Lehtilä and Strauss 1999, Barber et al. 2011). Herbivores may also influence fitness indirectly, for example, by causing flowers to have a degraded appearance that results in lower rates of pollination (Karban and Strauss 1993, Mothershead and Marquis 2000, Hambäck 2001, Suárez et al. 2009, Jordan and Harder 2006, Halpern et al. 2010). Furthermore, induced chemical defenses found in pollinator rewards (pollen and nectar) can alter pollinator visitation (Irwin et al. 2004).

Research that examines the outcome of the effect of more than one herbivore species

on a system is important as it sheds light on the effects of the herbivores in each other's presence and the potential interactions between them (Morris et al. 2007). Study systems include plant populations with spatially-segregated herbivores, (i.e., above and below-ground herbivores on the same plant species) (Grange and Brown 1989, Nötzold et al. 1998, Maron 1998, Brown and Grange 1990, Masters et al. 2001, Poveda et al. 2003, Hunt- Joshi 2004, Barber et al. 2012), or alternatively, herbivores that share plant resources (Myers 1985, Meyer 1993, Blossey 1995a, 1995b, Denno et al. 1995, Myers et al. 2009). There is a growing body of research to suggest that the presence of one herbivore can impact the way that another herbivore affects a host plant (Grange and Brown 1989, Nötzold et al. 1998, Poveda et al. 2003, Strauss et al. 2006). Biological control programs introduce more than one species of herbivore in the hopes that the biological control agents will either operate synergistically (Ehler and Hal 1982, Wilson et al. 2004), or at least increase the likelihood of getting the 'right' agent into the system (Myers 1985, Denoth et al. 2002).

This study explores the independent effects of two above-ground, herbivorous biological control agents, as well as their potential interactions, on the growth and reproduction of the invasive plant purple loosestrife (*Lythrum salicaria*, hereafter *Lythrum*), in addition to the diversity of the surrounding plant community. The findings of this study shed light on the degree to which the Ontario biological control program has contributed to the management of *Lythrum*, which is known to be economically and environmentally costly, as well providing tools to improve the general understanding of biological control effectiveness and the ecological effects of species introductions.

Study Species

Lythrum was introduced to North America from Eurasia in the early 1800's (Stuckey

1980). On several occasions, likely through ballast waters sourced from European marshes, *Lythrum* plant roots and seeds were unloaded into North American harbors (Stuckey 1980, Thompson et al. 1987). These multiple introductions could have increased the available genetic variation (Dlugosch and Parker 2008, Jones and Gomulkiewicz 2012), potentially contributing to *Lythrum*'s successful establishment and invasion of North America. *Lythrum* plants spread along rivers and smaller waterways, sometimes establishing very dense stands. *Lythrum* was originally reported to have the potential to cause serious and negative effects on wetlands due to its capacity to alter landscapes and reduce biodiversity (Stuckey 1980, Blossey and Skinner 2001). *Lythrum* reproduces both asexually and sexually, capable of producing millions of seeds per plant (Thompson et al. 1987). These highly productive plants can produce several stems of up to 2.7 m in height (Thompson et al. 1987, Mal et al. 1992, Blossey 1995a), over a kilogram of biomass and 100 stems per m^2 (Albright 2004) and inflorescences can cumulatively measure over 1000 cm per plant (Blossey and Schat 1997). The dense perennial rootstock and essentially unlimited seed bank (Yakimowski et al. 2005, Myers and Risley 1999), in addition to the thick layer of dead stems that can persist undecomposed for years, prevent the regrowth of other plant species (Grout et al. 1997, Blossey and Hunt-Joshi 2003). This combination of traits contributes to the formation of dense monotypic *Lythrum* stands and makes its eradication virtually impossible and its management an ongoing challenge.

The actual extent of the impacts of *Lythrum* on North American ecosystems remains unclear (reviewed in Corrigan 2006, Farnsworth and Ellis 2001). Some research suggests that it can easily out-compete native wetland plants (Stuckey 1980, Gaudet and Keddy 1988, Mal et al. 1997, Weihe and Neely 1997), reducing species diversity (Thompson et al. 1987, Blossey and Skinner 2001, Hovick et al. 2011). Other research shows that *Lythrum* does not

have a competitive advantage with respect to native plants (Houlahan and Findlay 2004, Denoth and Myers 2007) and that its presence has no impact on species diversity (Treberg and Husband 1999, Hager and Vinebrooke 2004, and reviews by Anderson 1995, Hager and McCoy 1998, Lavoie 2010).

Prior to the 1990s, various eradication and management strategies, including the use of fire, herbicides, mowing, flooding and mechanical removal were used against *Lythrum* in North America (Malecki and Rawinski 1985, Thompson et al. 1987, Malecki et al. 1993). When these failed, or were deemed unsustainable, an alternative approach was sought. A North American biological control program for *Lythrum* was initiated in the early 1990's with the approval of two leaf beetles, *Galerucella calmariensis* and *G. pusilla*, along with two weevils, the root-feeder *Hylobius transversovittatus* and the flower-feeding *Nanophyes marmoratus* (Lindgren et al. 2002). The expectation was that the separate species would feed on distinct portions of the plant, thereby maximizing management capacity (Wilson et al. 2004, Blossey 1995, Blossey and Schroder 1993). Within a year of approval, the biological control agents were released in different quantities and combinations in *Lythrum* invaded communities all over North America (Blossey 1993, Malecki et al.1993, Hight et al. 1995, Blossey, Skinner and Taylor 2001). In Ontario, the two *Galerucella* species were released on several occasions and in great numbers (Figure 1.1) along with a few releases of the root-feeding weevil (not thought to have established) (Corrigan et al. 1998, Lindgren et al. 2002) as part of a biological control program initiated by the University of Guelph and the provincial government (Ontario Ministry of Natural Resources) (Malecki et al. 1993, Hight et al. 1995, Blossey et al. 2001).

All life stages of *Galerucella calmariensis* (herafter *Galerucella*) are easily spotted. This species is the more successful of the two released species of *Galerucella*, and thus most

commonly encountered, and the only one present in my sites. Adults emerge in the spring after overwintering in the leaf litter in *Lythrum* populations. They mate and lay eggs in groups of 5-7 on *Lythrum* leaves (Blossey 1995). The first larval instars often feed on the meristematic tissue of the plant's apex, halting that stem's terminal growth and inducing branching (Corrigan et al. 1998, Schat and Blossey 2005). Later instars feed on young and mature leaves, as well as the outer layers of the stem. *Galerucella* pupate in the leaf litter, the adults arriving 30-40 days after eggs are seen. These leaf feeders are known to reduce the plant's photosynthetic area, performance, height, stem and root biomass (Blossey and Schat 1997, Landis et al. 2003) and seed production (Katovich et al. 2001). At very high levels (≥ 2 larvae/ cm stem) *Galerucella* can completely defoliate plants (Blossey 1995). Their tendency to aggregate facilitates mate and/or host finding (Grevstad and Hertzig 1997). However, because of this tendency, it is common to find *Galerucella* on less than half of *Lythrum* plants in a population, even in heavily populated sites (Blossey 1995, McAvoy et al. 1997, Dech and Nosko 2001). Although *Galerucella* tend to be fairly sedentary (Albright 2004), they have also been reported to disperse long distances (Grevstad and Hertzig 1997, Boag and Eckert 2013).

Nanophyes marmoratus (herafter *Nanophyes*) were never officially released in Ontario (Lindgren et al. 2002). This was largely due to the fact that they had not been available early on in the history of the program, and once available the program had already begun showing signs of success with *Galerucella* beetles (J. Corrigan pers. comm.). In areas where *Nanophyes* have been released (the closest being in the northeastern United States and Manitoba (Lindgren et al. 2002, VanDriesche 2002)), they are generally known to successfully establish and be strong dispersers, often spreading in a northeastern pattern, facilitated by the prevailing winds (Ferrarese and Garono 2010). Presumably for these

reasons, *Nanophyes* can now be found in several sites in Ontario and Quebec (Douglas 2013, St. Louis 2013), where they were never officially released.

Nanophyes is an easily identified weevil. It is univoltine and overwinter as adults, appearing on *Lythrum* as early as late May in Ontario populations. These specialist weevils feed on the youngest leaves in a distinct pattern, but move to the flower buds as soon as possible. Here, they mate and feed on the inner parts of the developing flower (Blossey and Schroder 1993). Towards the end of June and for the rest of the summer, eggs are laid singly into the closed flower buds. The larvae eat the stamens and often the petals and ovary. These buds do not flower but become filled with frass and are eventually aborted (Blossey and Schroder 1993). When the second-generation adults appear in the late summer, they may feed on foliage before overwintering.

Research Questions

The main objective of my thesis was to investigate the impacts of the original biological control agent, *Galerucella*, on individual *Lythrum* plants and populations by assessing the plants and their associated plant communities in several sites that have been exposed to a range of herbivore activity. In monitoring the selected sites, the frequently detected *Nanophyes* was noted and subsequently included in the project. This has given me the opportunity to look at both herbivore species, their impacts on the target host, as well as possible interactions they may have with one another. Specifically I asked:

1) Given that herbivory is often associated with a reduction in plant biomass and height, and since *Galerucella* is known to feed primarily on developing leaves and shoots, do we see an impact of *Galerucella* present day density and /or colonization history on *Lythrum* **productivity** (plant height, biomass and leaf tissue)?

2) Given that the removal of leaves and plant reproductive structures via herbivory can reduce overall fitness and that these herbivores (*Galerucella* and *Nanophyes*) are associated with feeding on leaves and reproductive tissues of *Lythrum*, do we see an impact of either the density and/ or colonization history of these herbivores on the **reproductive output** (i.e., inflorescence length and fruit production) of *Lythrum*?

3) Given that *Lythrum* is known as a strong competitor for space, light, pollinators and other key plant resources, and that the presence of herbivores tends to reduce the competitive ability of invasive plant species, do we do we see an impact of *Galerucella* present day density and/ or its colonization history on the 'recovery' of the plant community (i.e., plant community **species richness**)?

References

Albright M.F, W.N. Harman, S.S. Fickbohm, H. Meehan, S. Groff, and T. Austin. 2004. Recovery of native flora and behavioural responses of *Galerucella sp*. following biocontrol of purple loosestrife. The American Midland Natualist. **152**:248-254.

Anderson, M. G. 1995. Interactions between *Lythrum salicaria* and native organisms: A critical review. Environmental Management **19**:225-231.

Barber, N. A., L. S. Adler, and H. L. Bernardo. 2011. Effects of above- and belowground herbivory on growth, pollination, and reproduction in cucumber. Oecologia **165**:377-386.

Barber, N. A., L. S. Adler, N. Theis, R. V. Hazzard, and E. T. Kiers. 2012. Herbivory reduces plant interactions with above- and belowground antagonists and mutualists. Ecology **93**:1560-1570.

Bellows, T.S., 2001. Restoring population balance through natural enemy introductions. Biological Control **21**:199–205.

Bennett A.E., M. Thomsen and S. Y. Strauss. 2011. Multiple mechanisms enable invasive species to suppress native species. American Journal of Botany **98**:1086-94.

Bjerknes A, O. Totland, S. Hegland and A. Nielsen. 2007. Do alien plant invasions really affect pollination success of native plant species? Biological Conservation **138**:1-12.

Blossey, B. and R. Nötzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: A hypothesis. Journal of Ecology **83**:887-889.

Blossey, B., D. Schroeder, S. D. Hight, and R. A. Malecki. 1994a. Host specificity and environmental impact of the weevil Hylobius transovittatus, a biological control of purple loosestrife (*Lythrum salicaria*). Weed Science **42**:128-133.

Blossey, B., D. Schroeder, S. D. Hight, and R. A. Malecki. 1994b. Host specificity and environmental impact of two leaf beetles (*Galerucella calmariensis* and *G. pusilla*) for biological control of purple loosestrife (*Lythrum salicaria*). Weed Science **42**:134-140.

Blossey, B. and M. Schat. 1997. Performance of *Galerucella calmariensis* (Coleoptera: Chrysomelidae) on different North American populations of purple loosestrife. Environmental Entomology **26**:439-445.

Blossey, B., L. C. Skinner, and J. Taylor. 2001. Impact and management of purple loosestrife (*Lythrum salicaria*) in North America. Biodiversity and Conservation **10**:1787-1807.

Boag A.E. and C.G. Eckert. 2013. The effect on host abundance on the distribution and impact of biocontrol agents on purple loosestrife. Bioscience **20**:90-99.

Brody, A. K. 1997. Pollinators, herbivores and seed predators on flowering phenology.

Ecology. **78**: 1624-1631.

Brown V. K. and A. C. Grange. 1989. Differential effects of above-and belowground insect herbivory during early plant succession. Oikos. **54**:67-76.

Brown V. K. and A. C. Grange. 1990. Insect herbivory below-ground. Advances in Ecological Research **20**:1-58.

Brown B. J. and R. J. Mitchell. 2001. Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. Oecologia **129**:43-9.

Carson W. P., S. M. Hovick, A. J. Baumert, D.E. Bunker and T. H. Pendergast. 2008. Evaluating the post-release efficacy of invasive plant biocontrol by insects: a comprehensive approach. Arthropod-plant interactions **2**:78-86.

Chun Y., M. L. Collyer, K. Moloney, and J. Nason. 2007. Phenotypic plasticity of native vs. invasive purple loosestrife: a two state multivariate approach. Ecology **88**:1499-512.

Colautti R, S. Bailey S, C. van Overdijk, K. Amundsen, H. MacIsaac. 2006. Characterised and Projected Costs of Nonindigenous Species in Canada. Biological Invasions **8**:45-59.

Cory, J. S. and J. H. Myers. 2000. Direct and indirect ecological effects of biological control. Trends in Ecology and Evolution **15**:137-139.

Corrigan J. E., D. L. Mackenzie and L. Simser.1998. Field observations of non-target feeding by *Galerucella calmeriensis*, an introduced biological control agent of purple loosestrife, *Lythrum salicaria*. Proceedings of the Entomological Society of Ontario. **129**:99-106.

Corrigan, J. 2006. University of Guelph researched and then produced a document entitled ‘Ten Years After’ – A Summary of Results from the Ontario Purple Loosestrife Biological Control Programs for the Ontario Ministry of Natural Resources (OMNR). (unpublished)

Corrigan, J., Gillespie, D. R., De Clerck-Floate, R. and Mason, P. G. 2013. *Lythrum salicaria* L., Purple Loosestrife (Lythraceae) Pages 363–366 *in* P. G. Mason D. R. Gillespie, editors. Biological Control Programmes in Canada 2001–2012. CABI Publishing, Wallingford, Oxfordshire, UK.

Crawley, M. J. 1986. The population biology of invaders. Philosophical Transactions of the Royal Society of London B: Biological Sciences. **314**:711-731.

Crawley, M. J. 1989. Insect herbivores and plant populations dynamics. Annual Review of Entomology **34**:531-564.

Debach P. and D. Rosen 1991. Biological Control by Natural Enemies. 2nd Ed. Cambridge University Press. Cambridge, UK.

Dech J. P. and P. Nosko. 2001. Population establishment, dispersal and impact of *Galerucella pusilla* and *G. calmeriensis* introduced to control purple loosestrife in central Ontario. Biological Control **23**:288-236.

Denno, R. E., M. S. McClure, and J. R. Ott. 1995. Interspecific interactions in phytophagous insects: competition re-examined and resurrected. Annual Review of Entomology **40**:297-331.

Denoth, M., L. Frid and J. H. Myers. 2002. Multiple agents in biocontrol: improving the odds? Biological Control **24**:20-30.

Denoth, M. and J. H. Myers. 2007. Competition between *Lythrum salicaria* and a rare species: Combining evidence from experiments and long-term monitoring. Plant Ecology **191**:153-161.

Dlugosch K and I. Parker. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology **17**:431-49.

Doak, D. F. 1992. Lifetime impacts of herbivory for a perennial plant. Ecology **73**:2086-2099.

Doody, J. S., B. Green, D. Rhind, C. M. Castellano, R. Sims, and T. Robinson. 2009. Population-level declines in Australian predators caused by an invasive species. Animal Conservation **12**:46–53.

Ehler, L.E. and R.W. Hall. 1982. Evidence for competitive exclusion of introduced natural enemies in biological control. Environmental Entomology **11**:1–4.

Farnsworth, E. and D. Ellis. 2001. Is purple loosestrife (*Lythrum salicaria*) an invasive threat to freshwater wetlands? Conflicting evidence from several ecological metrics. Wetlands **21**:199-209.

Ferguson, K.I and P. Stiling. 1996. Non-additive effects of multiple natural enemies on aphid populations. Oecologia **108**:375–379.

Ferrarese, E., and R.J. Garono. 2010. Dispersal of *Galerucella pusilla* and *G. calmariensis* via passive water dispersal in the Columbia River Estuary. Biological Control **52**:115-122.

Gaudet, C. L. and P. A. Keddy. 1988. A comparative approach to predicting competitive ability from plant traits. Nature **334**:242-243.

Gange, A. C. 1990. Effects of insect herbivory on herbaceous plants. Pages 49-62 *in* J. J. Burdon and S. R. Leather, editors. Pests, pathogens, and plant communities. Black-well Scientific, Oxford, UK.

GrangeA.C. and V.K. Brown. 1989. Effects of root herbivory by and insect on a foliar species,

mediated through changes in a host plant. Oecologia 81:38-42.

Hager, H. A. and K. D. McCoy. 1998. The implications of accepting untested hypotheses: A review of the effects of purple loosestrife (*Lythrum salicaria*) in North America. Biodiversity and Conservation **7**:1069-1079.

Hager, H. A. and R. D. Vinebrooke. 2004. Positive relationships between invasive purple loosestrife (*Lythrum salicaria*) and plant species diversity and abundance in Minnesota wetlands. Canadian Journal of Botany **82**:763-773.

Halpern, S. L., L. S. Adler, and M. Wink. 2010. Leaf herbivory and drought stress affect floral attractive and defensive traits in *Nicotiana quadrivalvis*. Oecologia **163**:961-971.

Hambäck, P. A. 2001. Direct and indirect effects of herbivory: Feeding by spittlebugs affects pollinator visitation rates and seedset of *Rudbeckia hirta*. Ecoscience **8**:45-50.

Headrick, D.H. and R.D. Goeden. 2001, Biological control as a tool for ecosystem management. Biological Control **21**: 249-257

Hovick, S. M., D. E. Bunker, C. J. Peterson, and W. P. Carson. 2011. Purple loosestrife suppresses plant species colonization far more than broad-leaved cattail: Experimental evidence with plant community implications. Journal of Ecology **99**:225-234.

Houlahan J.E. and C.S. Findlay 2004. Effect of invasive plant species on temperate wetland plant diversity. Conservation Biology **18**:1132-1138.

Howarth, F.G. 1983. Classical biological control: panacea or Pandora's box. Proceedings of the Hawaiian Entomological Society **24**:239–244.

Hunt-Joshi, T. R., B. Blossey, and R. B. Root. 2004. Root and leaf herbivory on *Lythrum salicaria*: Implications for plant performance and communities. Ecological Applications **14**:1574-1589.

Irwin, R.E. L.S. Adler and A. K. Brody. 2004. The dual role of floral traits: pollinator attraction and plant defense. Ecology **85**:1503–1511.

Jensen K.I.N., P. Harris and M.G Sampsom. 2002. *Hypericum perforatum*L. St. John's Wort (Cluiasceae). Pages 361-368 *in* P. G. Mason and J. T. Huber, editors. Biological Control Programmes in Canada, 1981-2000. CABI Publishing, Wallingford, UK.

Jones E. Ii and R. Gomulkiewicz. 2012. Biotic interactions, rapid evolution and the establishment of introduced species. The American Naturalist. **179**:E28-E36.

Jordan C. and L. Harder. 2006. Manipulation of bee behavior by inflorescence architecture and its consequences for plant mating. The American Naturalist **167**:496-509.

Karban, R. and S. Y. Strauss. 1993. Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. Ecology **74**:39-46.

Katovich, E. J. S., D. W. Ragsdale, L. C. Skinner, and R. L. Becker. 2001. Effect of *Galerucella* spp. feeding on seed production in purple loosestrife. Weed Science **49**:190-194.

Keane, R. M. and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology and Evolution **17**:164-170.

King V.M. and R.D. Sargent. 2012. Presence of an invasive species alters pollinator visitation to a native. Biological Invasions **14**:1809-18.

Landis, D. A., D. C. Sebolt, M. J. Haas, and M. Klepinger. 2003. Establishment and impact of *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae) on *Lythrum salicaria* L. and associated plant communities in Michigan. Biological Control **28**:78-91.

Lau J.A. 2008. Beyond the ecological: biological invasions alter natural selection on a native plant species. Ecology **89**:1023-31.

Lavoie, C. 2010. Should we care about purple loosestrife? The history of an invasive plant in North America. Biological Invasions **12**:1967-1999.

Lawton, J. H. 1983. Plant architecture and the diversity of phytophagous insects. Annual Review of Entomology. **28**:23-39

Lawton, J. H. and K. C. Brown. 1986. The population and community ecology of invading insects. Philosophical Transactions of the Royal Society of London B: Biological Sciences **314**:607-617.

Lehtilä, K. and S. Y. Strauss. 1997. Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. Oecologia **111**:396-403.

Lehtilä, K. and S. Y. Strauss. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. Ecology **80**:116-124.

Lindgren, C. J., J. Corrigan and R. A. De Clerck-Floate. 2002. *Lythrum salicaria* L., purple loosestrife (Lythraceae). Pages 383-390 *in* P. G. Mason and J. T. Huber, editors. Biological Control Programmes in Canada, 1981-2000. CABI Publishing, Wallingford, UK.

Louda, S.M., R.W. Pemberton, M.T. Johnson and P. A. Follett, P. A. 2003. Nontarget effects – the Achilles' Heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. Annual Review of Entomology **48**:365-396.

McAvoy T.J., L.T Kok and W.T Mayes. 1997. Phenology of an established population of *Galerucella calmeriensis* (L.) and *Galerucella pusilla* (Duft.) (Coleoptera: Chrysomelida) on purple loosestrife, *Lythrum salicaria* L. (lythraceae), in Southwest Virginia. Biological Control **9**:106-111.

Mack, R. N., D. Simberloff, W. M. Londsdale, H. Evans, M. Clout and F. A. Bazzaz. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. Ecological Applications **10**:689-710.

Mal, T. K., J. Lovett-Doust, and L. Lovett-Doust. 1997. Time-dependent competitive displacement of *Typha angustifolia* by *Lythrum salicaria*. Oikos **79**:26-33.

Malecki, R. A. and T.J. Rawinski. 1985. New methods for controlling purple loosestrife (*Lythrum salicaria*). New York Fish and Game Journal. **32**:9-19.

Malecki, R. A., B. Blossey, S. D. Hight, D. Schroeder, L. T. Kok, and J. R. Coulson. 1993. Biological control of purple loosestrife. BioScience **43**:680-686.

Maron J.L.1998. Insect herbivory above and below ground: individual and joint effect on the plant fitness. Ecology **79**:1281-1293.

Mason P. and J. Huber. 2001. Biological Control Programmes in Canada 1981-2000. CABI Publishing, Wallingford, UK.

Mason P. and D. Gillespie 2013. Biological Control Programmes in Canada 2001-2012. CABI Publishing, Wallingford, UK.

Mason P. G. and J. T. Huber, editors. Biological Control Programmes in Canada, 1981-2000. CABI Publishing, Wallingford, UK.

Masters G.J., T. Hefin Jones and M. Rogers. 2001. Host-plant mediated effects of root herbivory on insect seed predators and their parasites. Oecologia. **127**:246-20.

McEvoy P., C. Cox and E. Coombs. 1991. Successful biological control of ragwort, *Senecio jacobaea*, by introduced insects in Oregon. Ecological Applications **1**:430-442.

Meyer, G. A. 1993. A comparison of the impacts of leaf- and sap-feeding insects on growth and allocation of goldenrod. Ecology **74**:1101-1116.

Miller, T. E. and J. Travis. 1996. The evolutionary role of indirect effects in communities. Ecology **77**:1329-1335.

Mothershead, K. and R. J. Marquis. 2000. Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. Ecology **81**:30-40.

Morris, W. F., R. A. Hufbauer, A. A. Agrawal, J. D. Bever, V. A. Borowicz, G. S. Gilbert, J.L. Maron, C. E. Mitchell, I. M. Parker, G. P. Alison, M. E. Torchin, and D. P. Vázquez. 2007. Direct and interactive effects of enemies and mutualists on plant performance: A meta-analysis. Ecology **88**:1021-1029.

Müller-Schärer, H and V. K. Brown. 1995. Direct and Indirect Effects of above- and Below-Ground Insect Herbivory on Plant Density and Performance of *Tripleurospermum perforatum* during Early Plant Succession. Oikos **72**:36-41.

Müller-Schärer H, U. Schaffner and T. Steinger. 2004. Evolution in invasive plants: implications for biocontrol. Trends in Ecology and Evolution **19**:417-22.

Mutikainen, P. and L. F. Delph. 1996. Effects of herbivory on male reproductive success in plants. Oikos **75**:353-358.

Myers, J. 1985. How many insect species are necessary for successful biological control of weeds? Pages 77-82 *in* E. S. Delfosse, editor. Proceedings of the VI Symposium on the Biological Control of Weeds (Vancouver). Agriculture Canada, Ottawa, Canada.

Myers, J.H., C. Jackson, H. Quinn, S.R. White and J.S. Cory. 2009. Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canada. Biological Control **50**:66-72.

Myers, J.H. and C. Risley. 2000. Why reduced seed production is not necessarily translated into successful biological weed control. Pages 569–581 *in* N. Spencer, editor. Proceedings X. International Symposium Biological Control of Weeds. Montana State University, Bozeman, MO.

Nötzold, R., B. Blossey, and E. Newton. 1998. The influence of below ground herbivory and plant competition on growth and biomass allocation of purple loosestrife. Oecologia **113**:82-93.

Paynter, Q., J.M. Overton, R.L. Hill, S.E. Bellgard, M.I. Dawson. 2012. Plant traits predict the success of weed biocontrol. Journal of Applied Ecology **49**:1140-1148.

Pearson, D.E., and Callaway, R.M. 2003. Indirect effects of host-specific biological control agents. Trends in Ecology and Evolution **18**:456-461.

Pellegrino, G. and A. Musacchio. 2006. Effects of defoliation on reproductive success in two orchids, *Serapias vomeracea* and *Dactylorhiza sambucina*. Annales Botanici Fennici **43**:123-128.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics **52**:273-288.

Poveda, K., I. Steffan-Dewenter, S. Scheu, and T. Tscharntke. 2003. Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set. Oecologia **135**:601-605.

Poveda, K., I. Steffan-Dewenter, S. Scheu, and T. Tscharntke. 2005. Effects of decomposers and herbivores on plant performance and aboveground plant-insect interactions. Oikos **108**:503-510.

Quesada, M., K. Bollman and A. G. Stephenson. 1995. Leaf damage decreases pollen production and hinders pollen performance in *Cucurbita texana*. Ecology **76**:437- 443.

Rausher, M. D. and P. Feeny. 1980. Herbivory, plant density, and plant reproductive success: The effect of *Battus philenor* on *Aristolochia reticulata*. Ecology **61**:905-917.

Ruesink J. L., I. M. Parker, M. J Groom and P. M. Kareiva.1995. Reducing the risks of nonindigenous species introductions. Bioscience **45**:465-477.

Schat, M. and B. Blossey. 2005. Influence of natural and simulated leaf beetle herbivory on biomass allocation and plant architecture of purple loosestrife (*Lythrum salicaria* L.). Environmental Entomology **34**:906-914.

Secord, D. and P. Kareiva, P. 1996. Perils and pitfalls in the host specificity paradigm. Bioscience **46**:448-453.

Simberloff, D. 2011. Risks of biological control for conservation purposes. BioControl **57**: 263-276.

Simberloff D. and P. Stiling. 1996. How risky is biocontrol? Ecology **77**:1965-74.

Stastny M., U. Schaffner and E. Elle. 2005. Do vigour of introduced populations and escape form specialist herbivores contribute to invasiveness? Journal of Ecology **93**:27-37.

Strauss, S. Y. 1991. Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. Ecology **72**:543-558.

Strauss, S. Y. and R. E. Irwin. 2004. Ecological and evolutionary consequences of multispecies plant-animal interactions. Annual Review of Ecology, Evolution, and Systematics **35**:435-466.

Strauss, S. Y., J. A. Lau and S. P. Carol. 2006. Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? Ecology Letters **9**:357-374.

St. Louis, E. 2013. New Ontario records for *Nanophyes m. marmoratus* (Goeze 1777) (Coleoptera: Brentidae) introduced into North America for the classical biological control of purple loosestrife. Journal of the Entomological Society of Ontario. **144**: 19-123.

Stuckey, R. L. 1980. Distributional history of *Lythrum salicaria* (purple loosestrife) in North America. Bartonia **47**:3-20.

Suárez, L. H., W. L. Gonzalez, and E. Gianoli. 2009. Foliar damage modifies floral attractiveness to pollinators in *Alstroemeria exerens*. Evolutionary Ecology **23**:545-555.

Swope, S. M. and I. M. Parker. 2010. Trait-mediated interactions and lifetime fitness of the invasive plant *Centaurea solstitialis*. Ecology **91**:2284-2293.

Swope, S. M. and I. M. Parker. 2012. Complex interactions among biological control agents, pollinators, and an invasive weed: A structural equation modeling approach. Ecological Applications **22**:2122-2134.

Syrett, P., D.T. Briese and J.H. Hoffman. 2000. Success in biological control of terrestrial weeds by arthropods. Pages 189-230 *in* G.Gurr and S. Wratten, editors. Biological Control: Measures of Success. Kluwer Academic Publishers.

Thompson, D. W., R. L. Stuckey, and E. B. Thompson. 1987. Spread, impact and control of purple loosestrife (*Lythrum salicaria*) in North American wetlands. Fish and Wildlife Research 2. U.S. Department of the Interior, Fish and Wildlife Service, Washington D.C.

Thompson F. L. and C.G. Eckert. 2004. Trade-offs between sexual and clonal reproduction in an aquatic plant: experimental manipulations vs. phenotypic correlations. Journal of Evolutionary Biology **17**:581-92.

Treberg, M. and B. Husband. 1999. Relationship between the abundance of *Lythrum salicaria* (purple loosestrife) and plant species richness along the Bar River, Canada.Wetlands **19**:118-125.

Van Driesche, R., B. Blossey, M. Hoddle, S. Lyon and R. Reardon, R. 2002. *Biological control of invasive plants in the Eastern United States*. USDA Forest Service FHTET-2002-04. 413 pp.

van Kleunen, M., W. Dawson, D. Schlaepfer, J. M. Jenschke. 2010. Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. Ecology Letters **13**:947-58.

van Lenteren J. C., J. Bale, F. Bigler. H. M. T. Hokkanen and A. J. M. Loomans. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. Annual Review of Entomology. **51**:609-634.

Vranjic, J. A. and P. J. Gullan. 1990. The effect of a sap-sucking herbivore, *Eriococcus coriaceus* (Homoptera: Eriococcidae), on seedling growth and architecture in *Eucalyptus blakelyi*. Oikos **59**:157-162.

Weihe, P. E. and R. K. Neely. 1997. The effects of shading on competition between purple loosestrife and broad-leaved cattail. Aquatic Botany **59**:127-138.

Williamson, M. 1996. Biological Invasion. Chapman & Hall. T.J. Press, Padstow, UK.

Williamson, M. and A. Fitter. 1996. The varying success of invaders. Ecological Society of America. **77**:1661-1666.

Wilson, L. M., M. Schwarzlaender, B. Blossey and C. B. Randall. 2004. Biology and biological control of purple loosestrife. USDA Forest Service, FHTET-04-12.78.

Wolfe, L. M. 2002. Why alien invaders succeed: Support for the escape-from-enemy hypothesis. The American Naturalist **160**:705-711.

Zangerl, A. R., J. G. Hamilton, T. J. Miller, A. R. Crofts, K. Oxborough, M. R. Berenbaum, and E. H. de Lucia. 2002. Impact of folivory on photosynthesis is greater than the sum of its holes. Proceedings of the National Academy of Sciences of the United States of America **99**:1088-1091.

CHAPTER 2: An assessment of the effects of biological control on *Lythrum salicaria:* implications for plant performance (productivity and reproductive output) and community species richness.

Introduction

Since exotic species are now being moved around the world more frequently than ever, understanding their potential consequences is key. Each introduction has the potential to result in serious economic and ecological consequences (e.g., Scotch broom, Kudzu vine, Emerald ash borer, Asian longhorned beetle, Asian carp, and Zebra mussels, to list a few of the more notorious cases). The effect of invasive, alien species on community biodiversity has often been discussed and debated (Thompson et al. 1987, Mack et al. 2000, Levine et al. 2003, Myers and Bazely 2003, Davies et al. 2005, Melbourne 2007, Gaertner et al. 2009, Pimentel 2005, Strauss et al. 2006, Thomas and Reid 2007, Gaertner et al. 2009, Powell et al. 2013). While most introduced species are likely innocuous (Davis 2003, Gurevitch and Padilla 2004), those that do become invasive species can be the direct cause or 'drivers' of biodiversity decline (Gordon 1998, Wilcove et al. 1998, Mack et al. 2000).

The invasiveness of a species depends on a variety of factors. Species that are introduced multiple times are more likely to transition from 'introduced' to 'invasive', possibly because there is a greater amount of genetic diversity available to enable the species to adapt to local conditions (Gaertner et al. 2009, Jones and Gomulkiewicz 2012). Furthermore, outside of its native range, a species may not be exposed to as many predator or herbivore species, which may allow it to multiply and spread more rapidly than native species (Keane and Crawley 2004, Stastny et al. 2005, Jogesh et al. 2008).

The use of classical biological control against invasive plants typically involves the introduction of specific known herbivores from the invasive plant species' native range. This is a strategy that has been applied as a management practice in North America for decades (Crawley 1989, McFayden 1998, Mason and Huber 2001, Pearson and Callaway 2003, Mason and Gillespie 2013), in spite of the inherent risks involved (Howarth 1983, Secord and Kareiva 1996, Cory and Myers 2000, Simberloff and Stiling 2006, Carson et al. 2008, Simberloff 2011).

Herbivores (including foliage, flower and seed feeders), can have multiple negative impacts on plant performance (Rausher and Feeny 1980, Louda et al. 1990, Karban and Strauss 1993, Mutikainen and Delph, 1996, Gronemeyer et al. 1997, Krupnick and Weis 1998, Lehtilä and Strauss 1999, Zamora et al. 1999, Strauss et al. 2001, Zangerl et al. 2002, Poveda et al. 2003 Strauss and Irwin 2004, Sharaf and Price 2004, Pellegrino and Musacchio 2006, Hladun et al. 2009). These impacts on performance can, in turn, have negative impacts on competitive ability, which can result in a reduction in competitive ability or depressed recruitment (Malecki et al. 1993, Jensen et al. 2002). When herbivory alters the architecture or size of a dominant plant in a community, there may also be indirect effects on the surrounding plant community (Crawley 1989, Carson and Root 2000).

A common management practice is the release of multiple niche-segregated herbivores at once, with the aim of reducing several aspects of an invasive plant's fitness, the hope being that the herbivores will act in an additive or synergistic manner (Harris 1985, Malecki et al. 1993, Blossey 1995, Strauss 1991, Wilson et al. 2004, Barber et al. 2012). Although pre-release research may provide adequate information to make informed decisions (Ehler 2000, Mason et al. 2013), post-release factors can align in unique or unexpected combinations, including those involving interactions among the introduced biological control

agents and other members of the ecosystem, including pollinators (Brody 1997, Lehtilä and Strauss 1997, Ohashi and Yahara 1998, Krupnick 1999, Mothershead and Marquis 2000, Suárez et al. 2009, Barber et al. 2011, Russell-Mercier 2013). Thus, it is important to ensure that post-release monitoring be included in biological control program, as it provides key information on the long term effects of the release(s), both from a biological control and an ecosystem integrity perspective (van Lenteren et al. 2006, Mason et al. 2013). Unfortunately, post-release monitoring is often neglected or under-funded (Ding et al. 2006, Mason et al. 2013).

The success of a biological control program can be assessed in several ways (Syrett et al. 2000), none of which are definitive. Some researchers have used a reduction in plant reproductive fitness (McClay and De Clerk-Floate 2002) or host plant density to quantify success (Huffaker and Kennett 1959), while others have applied the relatively higher standard of an associated return of native plant species or increased community diversity (reviewed in Farnsworth and Ellis 2001).

Overall, the 20 year-old Canadian biological control program for *Lythrum* has been considered a success (Lindgren et al. 2002), although such opinions are based on few actual quantitative studies (Myers and Denoth 1999, Denoth and Myers 2005, Dech and Nosko 2002), and/ or on unpublished evidence of *Galerucella sp.* behaviour and dispersal (Corrigan et al. 1998, Ali and Verbeek 1999, Corrigan et al. 2013). The results of the 'Ontario purple loosestrife programs' (which were mainly applied in the Southern part of the province) were summarized qualitatively ten years after the introduction of the beetles (Corrigan 2006). As the initial and subsequent releases of *Galerucella* sp. occurred on such a large scale and into populations without collecting rigorous information on the *Lythrum* plant traits or the surrounding communities, the ability to monitor their success was limited from the beginning.

Here I describe a study designed to assess both the effect of present-day density of the two biological control species *Galerucella* and *Nanophyes*, along with *Galerucella* colonization history, on the productivity and reproductive output of *Lythrum*, and the condition of the surrounding plant community. In particular, I was interested in the individual and combined impacts of the leaf-feeding beetle *Galerucella* and the flower-feeding weevil *Nanophyes*, on the height, above and below-ground biomass, inflorescence length and fruit production of *Lythrum*, in addition to the species richness of the plant communities in *Lythrum*-invaded sites. During early June, late July and late September 2013, I surveyed 18 eastern Ontario populations (Figure 2.1), chosen to represent a spectrum of *Galerucella* colonized populations, ranging from those that represent the earliest releases of *Galerucella* to populations that have never been exposed to *Galerucella.* Since *Nanophyes* was released in much lower numbers and far fewer locations in North America and because its post-release monitoring has been inconsistent, there are few reports that include anything about its spread or actual contribution to the management of *Lythrum* (e.g. Van Dreische et al. 2002, Piper et al. 2004, Ferrarese and Garono 2010, Yeates et al. 2012, St. Louis 2013). Unfortunately, because of this lack of information I was unable to also categorize the *Lythrum* populations used in my study according to their *Nanophyes* colonization history.

I predicted that increased exposure to *Galerucella* (through either beetle density and/or colonization history) would result in *Lythrum* plants exhibiting reduced above-ground biomass or altered biomass partitioning as well as reduced reproductive success (i.e., inflorescence length and fruit production). In addition, once I discovered the *Nanophyes* weevil in several of my sites, I became interested in studying its effects on *Lythrum*, and how those might interact with the presence of *Galerucella*. Finally, I assessed whether there is evidence to suggest that the introduction of biological control could result in a recovery of the

species richness of the invaded plant communities in my study.

My thesis work is designed to be a scientific assessment of the impacts of the biological control program established to for *Lythrum* in Ontario. Proper assessment is critical to determine whether the release program has met its goal of controlling the spread and impact of *Lythrum* in Ontario's wetland communities. Moreover, the presence of an unexpected, additional herbivore species in this system provides an opportunity to explore how the presence of multiple biological control agents (or this particular combination of species) has impacted the productivity and reproductive output of *Lythrum*.

Methods

Ontario's Lythrum Biological Control Program

Between 1992-1999, mating pairs of *Galerucella sp*. were released or redistributed into each of more than 200 heavily invaded *Lythrum* sites across Ontario (Figure 1.1). The releases were focused on the southwestern part of the province, with select northern and eastern sites (where my study was based). Over 320,000 beetles (of all life stages) were released in total (Corrigan et al. 1998, Lindgren et al. 2002, Corrigan 2006). Most of the Ontario releases were organized and well documented (such as those performed by the University of Guelph and Ontario Beetles), but others, made by various organizations and individuals, including Ducks Unlimited, the Ontario Federation of Anglers and Hunters (OFAH), private landowners and concerned citizens (Corrigan 2006), may not have been. Some release sites were quite isolated, reducing the likelihood of the beetle dispersing from the site. However, other release sites, such as those placed along waterways and/or roadsides, allowed beetles to migrate from the original release sites and into new and undocumented

Lythrum populations. In the vicinity of my study populations, only *G. calmeriensis* were released, and this was the only species of *Galerucella* detected in any of my study sites.

Lythrum plants are impacted early in the season with the emergence of adult *Galerucella* beetles (Figure 2.2a) who mate and lay eggs in groups of 5-7 on *Lythrum* leaves (Figure 2.2b) (Blossey 1995). The first larval instars feed primarily on new and meristematic stem tissue, halting that stem's vertical growth and inducing side branching and the production of secondary inflorescences (Malecki et al. 1993, Blossey et al. 1994, Hunt-Joshi et al. 2004, Schat and Blossey 2005). This change in architecture is associated with a delay in flowering (Schat and Blossey 2005) that can affect interactions between *Lythrum* and its pollinator community (Sharaf and Price 2004). Although *Galerucella* feeding can potentially increase the number of inflorescences, it can also lead to a decrease in the overall inflorescence length (Katovich et al. 2001, Schat and Blossey 2005), reducing the number of flowers (Lindgren 1999, Katovich et al. 2001, Landis et al. 2003), fruits and seeds produced (Katovich et al. 2001). Later instars feed on both young and mature leaves as well as on the outer layers of the stem and can defoliate entire plants at high densities (≥2 larvae / cm of shoot, Figure 2.2c), severely reducing plant photosynthetic area (Blossey and Schat 1997, Hunt 2002) as well as stem and root biomass (Blossey and Schat 1997, Dech and Nosko 2001).

The flower feeding weevil *Nanophyes* (Figure 2.2d, 2.2e) can now be found in more than 23 widely dispersed *Lythrum* populations in Ontario and Quebec (Douglas 2013, St. Louis 2013), although there were no documented releases in these provinces (Lindgren 2002). In fact, there are only three documented Canadian releases, all in southern Manitoba (1997) (Lindgren et al. 2002). When these weevils are released they are known to successfully establish and be strong dispersers capable of crossing large expanses of open water (Ferrarese

and Garono 2010), with the potential to disperse 100–300 km/year. *Nanophyes* found in our sites are likely a result of dispersal from the closest releases in upstate New York and other release sites in the northeastern United States (1994-2007) (Skinner 1996, Van Dreische et al. 2002). *Nanophyes* feed in the developing flowers, leading to a reduction in the number of flowers on an inflorescence (Figure 2.2f).

Study Sites

Galerucella releases described by D. McKenzie (pers. comm.) and J. Corrigan (OMNR 2006) were used to locate *Lythrum* populations that exhibited a range of herbivore exposure and/or colonization history. Herbarium records from the Canadian Museum of Nature and the Algonquin Provincial Park collections were used to locate additional populations of *Lythrum* with no recorded history of *Galerucella* exposure. During the summers of 2012 and 2013, a total of 24 sites in eastern Ontario (between Ottawa, Perth and Algonquin park) were visited in order to determine their suitability for the study. Subsequently, 18 sites were chosen (Figure 2.1) for the study based on their *Lythrum* population size and *Galerucella* activity. To the best of my ability, sites were chosen to minimize any association between geographic location and history of colonization. Selected sites were a minimum distance of nine kilometers from one another and were within 0.5 degrees of latitude (Table 2.1) from one another to minimize latitudinal variation in phenology (Montague et al. 2008). Herbarium records were used to confirm that the *Lythrum* populations chosen had existed for at least twenty years (most have existed for much longer).

Of the 18 *Lythrum* populations selected, 12 of them have a known number of years of *Galerucella* exposure (zero years or number of years from release date). Five sites were classified as '*Galerucella*-free' (GF), with no *Galerucella* or evidence of feeding damage found; seven of the sites were classified as 'early release' (ER) with a range of 16 to 22 years

of *Galerucella* exposure (Corrigan 2006, Norman et al. 2009); the remaining six were classified as 'intermediate colonization' (IC), having evidence of active *Galerucella* but not known as release sites (estimated at anywhere 1-15 years of *Galerucella* exposure). The categories GF, IC and ER are collectively referred to as 'colonization history'. The 18 sites also vary in terms of their present-day *Galerucella* density (Table 2.2) and damage. Not enough information on *Nanophyes* range or migration was available to do the equivalent ranking of site history, and so only the present day density of *Nanophyes* at each site was used as a variable in the analyses.

Data Collection

The goal of my study was to determine whether inter-population variation in *Galerucella* exposure (either present-day or historical) is associated with significant differences in the productivity (i.e., plant density, plant biomass) and reproductive success (i.e., flowering density, fruit production) of *Lythrum* plants and/or the condition of the surrounding community (i.e., species richness) of *Lythrum*-invaded communities in eastern Ontario.

Surveys were performed on two separate occasions during the summer of 2013, in early June and late July. I used a quadrat sampling method to measure *Lythrum* density and the species richness of the plant community at each of the 18 sites. At each visit, I set up three evenly spaced transect lines that ran longitudinally through the approximate center of the *Lythrum* population. Along each transect, I placed five 0.25 m^2 quadrats (0.5 m by 0.5 m) such that they were evenly spaced across the population. In each of the 15 quadrats, I counted and identified (to at least genus) all individual plants (using Peterson and McKenny1996, Newcomb 1977), including *Lythrum.* In addition, I measured the height as well as the number and the total length of all inflorescences on a haphazardly selected *Lythrum* stem within the

quadrat. I also personally assigned a semi-quantitative damage rating (0-5) (following Corrigan 2006, Grevstad 2006, Boag and Eckert 2013) to each stem. To determine present-day *Galerucella* and *Nanophyes* densities, adult beetles were counted first to minimize disturbance and flight, followed by egg masses and larvae (N.B. no *Nanophyes* eggs or larvae were detected during any of the sampling periods). Each beetle of each life stage was given a value of 1 in order to calculate total number of each herbivore species per stem. The presence of apical damage was also noted (following Blossey 1995a, Corrigan 2006). Although I recorded several other arthropod species on *Lythrum* (e.g., aphids, myrids, jewel beetles, other weevils, flea beetles and juvenile lepidopterans including leaf-rolling caterpillars, gypsy moth and wood nymphs) (as in Batra et al. 1986) in my study sites, none appeared to inflict visible damage and were not included in the study. In July, the procedure was repeated, however, as the plants were larger and more variable in size, the number of stems sampled was doubled (from 15 to 30), to obtain a more precise estimate of beetle density. Thus, I added five additional *Lythrum* stems per transect line, each located midway between adjacent quadrats.

In late September 2013, I collected twelve whole *Lythrum* plants from each site along evenly spaced longitudinal transect lines (as described above). Although it was not possible to collect plants from all sites on the same day due to travel time, all plants were bearing mature fruit at the time they were collected. I also measured the height and number of stems per plant at the time of collection. Once harvested, bagged and labeled, plants were returned to the laboratory, where I cleared the roots of loose, dry soil and thoroughly washed them in order to remove any clinging soil. Whole plants were then dried in paper bags in a drying oven at 72˚C for 24 hrs, as described in Bastolova and Kvet (2002). The dried roots, stems, leaves, and infructescences (fruiting stems), were divided up and weighed to an accuracy of 0.001 g, as described in Bastolova and Kvet (2002), in order to obtain dry-weight biomass measurements.

I also counted and measured the infructescences and recorded the number of attached fruit on each plant.

Response Variables

Plant productivity (Table 2.3) was measured as plant height (from ground level to the tallest part of the plant) and the above-ground biomass. The shoot to root ratios were calculated as the amount of above-ground biomass (stems, leaves and reproductive portions) relative to the below ground biomass. The density of *Lythrum* stems was also considered.

Variables that I have used to describe reproductive success include the percentage of plants flowering (plants were recorded as flowering if, during the July visit, the surveyed stem had at least one open flower), as well as the number and total lengths of inflorescences per cm of total plant height (in order to account for the inherent relationship between plant size, beetle density and flower production (Lande and Arnold 1983) (Table 2.4). For plants collected in September, I counted the number of fruit produced per plant and then calculated the number of fruit per gram of above-ground biomass (again, to account for relationships between plant size, beetle density and fruit production), as well as the number of fruit per cm of infructescence length, a variable I will hereafter refer to as 'fruit density'.

And finally, to assess the species richness of the surrounding plant community, I used the average quadrat species richness (Table 2.5) at each site as well as the total number of different species counted at each site. The number of plant families at each site is also included in the analyses, in order to consider the fact that while sites may be species rich, the richness may represent relatively few plant families.

Independent Variables

At each site, all *Galerucella* life stages per stem were added and averaged to obtain a site-specific estimate of *Galerucella* density, this was done during both the June and July

visits and these values were then averaged. Since *Nanophyes* adults were the only detectable life stage, the average number found per stem during the July visit have been used for the site-specific estimates of *Nanophyes* density (Table 2.2). The number of years since *Galerucella* was first introduced (based on release data) was used to express the duration of the exposure to the beetle at each site. For sites that were not originally part of the release program, I estimated the approximate duration of exposure using the year and distance (km) from the closest release site along with reported dispersal capability (9 km/yr) of *Galerucella*.

Colonization history and present-day *Galerucella* levels were also considered as categorical variables. The colonization history was divided into sites that were deemed '*Galerucella*-free' (GF), 'intermediate colonization' (IC) and 'early release' (ER). The sites were also categorized by their present-day *Galerucella* level: absent (0 *Galerucella* per stem), low (> 0, < 2 *Galerucella* per stem), and high (> 4 *Galerucella* per stem).

Statistical Analysis

All analyses were performed using the statistical software JMP Version 10 (SAS 2012). Unless indicated otherwise, 'site' is the unit of replication. Regression analyses were used to examine the relationship between the continuous variable of present day herbivore density (highly correlated with damage intensity (0-5), that was therefore omitted), and the site averages for several continuous plant traits. These plant traits, both measured and calculated, include: final plant height and biomass, the ratio of above to below-ground biomass, the percentage of flowering plants, the number and lengths of inflorescences per plant, number of fruit per plant as well as per gram of above-ground biomass (Table 2.3). One-way ANOVAs were used to explore relationships among selected response variables listed above and the two categorical variables for *Galerucella*, colonization history and

Galerucella density levels. Testing for significant difference between pairs was done using a Tukey-Kramer HSD test ($\alpha = 0.05$).

Using the July data set, three mixed models were developed to test for relationships between the density of the two species of biological control and the two reproductive response variables (Table 2.6). All models included three fixed effects: 'stem height' (known to be strongly correlated both response variables), the number of each of the two herbivores (*Galerucella* and *Nanophyes*) per stem (all continuous covariates) and 'site', which was treated as a random factor. Additional parameters that distinguished the models included an interaction term between the density of the two herbivores and an interaction term between *Galerucella* density and *Lythrum* stem height. Model coefficients and AICc were estimated using a restricted maximum likelihood (REML), mixed-modeling approach that allows for the inclusion of a 'random' factor. Using the Akaike Information Criteria (AICc), the best model (based on a compromise between number of parameters and explanatory power (Anderson et al. 2001)) of the three was selected.

Several of the response variables included in analyses (i.e. stem density, number of inflorescences and total length of inflorescences) were $\log_{10}$ transformed in order to meet the assumptions of parametric statistics (Quinn and Keough 2002).

Results

Site Characteristics

Geographical co-ordinates for the 18 sites are provided in Table 2.1, and a corresponding map is provided in Figure 2.1. Table 2.3 summarizes several *Lythrum* measurements for the 18 sites as means (± SE) and a table of Pearson correlation coefficients among them can be found in Table 2.5.

Nanophyes weevils were found at 14 sites and *Galerucella* beetles were found at 13 sites (Table 2.2). At the site level, the densities of the *Galerucella* adults and the *Nanophyes* adults are correlated ($F = 9.5787$, $df = 17$, $P = 0.0074$). However, this is not the case if the number of all *Galerucella* life stages (including egg masses and larva), are considered ($F = 2.6738$, $df = 17$, $P = 0.1215$). There was high variability in the number of leaf beetles and the number of weevils per stem, and, where present, herbivores were generally found on less than half of the stems surveyed in a population (average percentage of stems with *Galerucella*, 46% ± 10%, average percentage of stems with *Nanophyes*, 24% ± 4%). At the site level there was a strong correlation between mean *Galerucella* density and the mean damage rating ($F = 32.02$, $df = 17$, $P < 0.0001$) (as in Schooler and McEvoy 2006). Although I have primarily used *Galerucella* density (Table 2.2) in the analyses, these results may be compared to those of prior studies which use the damage scale (e.g., Corrigan 2006, Grevstad 2006, Boag and Eckert 2013). At no point did I find what Blossey and Schat (1997) refer to as 'breakout levels' of ≥ 2 *Galerucella* larvae/cm of stem.

Colonization history and present-day herbivore density

The number of years a site has been exposed to *Galerucella* was associated with higher mean *Galerucella* density ($F = 9.5548$, $df = 17$, $P < 0.007$) and damage rating ($F = 22.50$, $df = 17$, $P < 0.0002$) (Figure 2.3). When I expressed *Galerucella* exposure as a categorical variable I detected similar results (Figure 2.4). Early release sites ($n = 7$) had significantly higher mean *Galerucella* density ($F = 5.7340$, $df = 1, 16$, $P = 0.0292$) and damage ratings ($F = 10.33$, $df = 1, 16$, $P = 0.0054$) than those that were not early release sites (i.e., GF + IC, n = 11). When all three colonization categories are included in the model, associations between colonization history, *Galerucella* density ($F = 3.5922$, $df = 2, 15$, $P = 0.0531$) and damage rating ($F = 6.9250$, $df = 2, 15$, $P = 0.0074$) are clear.

Plant Productivity

The analyses for plant productivity are all at the site level, using the fall data. A positive trend was detected between mean stem height and both *Galerucella* density (F = 4.1413, df = 17, P = 0.0588) and *Nanophyes* density (F = 2.84, df = 17, P = 0.1112, Figure 2.5). A similar pattern, but with no significant association, was detected between the ($\log_{10}$) mean above-ground biomass and *Galerucella* density (F = 2.7961, df = 17, P = 0.1139) or *Nanophyes* density (F = 2.5732, df = 17, P = 0.1282). There was a negative trend between both the proportion of leaf biomass and *Galerucella* density (F = 3.1665, df = 17, P = 0.0942) as well as between the ratio of above to below-ground biomass and the densities of both *Galerucella* (F = 4.3487, df = 17, P = 0.0534), and *Nanophyes* (F = 3.9804, df = 17, P = 0.0634) (Figure 2.6). No association was detected between the density of *Lythrum* stems at a site and *Galerucella* (F = 1.5569, df = 17, P = 0.2301), or *Nanophyes* density (F = 2.4009, df = 17, P = 0.1408).

Reproductive Success

I detected a significant, positive relationship between *Galerucella* density and the number of inflorescences (per cm stem height), both at the site level where averages are used (Figure 2.7a, F = 6.3550, df = 17, P = 0.0227) as well as in the mixed-model approach (described above) where height is included as a fixed effect (Table 2.7). At the site level, there was no significant correlation between *Nanophyes* density and the mean number of inflorescences (per cm stem height), (Figure 2.7b, F = 1.9940, df = 17, P = 0.1771). Although a positive correlation is found between site *Galerucella* density and number of inflorescences, this does not translate into an associated increase in total length of inflorescences (per cm stem height) (Figure 2.7c, F = 0.0034, df = 17, P = 0.9545). Similarly, there is no correlation between *Nanophyes* density and total length of inflorescences (Figure 2.7d, F = 0.4815, df =

17, $P = 0.4977$) at the site level. However, in the mixed model, both the number of *Galerucella* per stem and the number of *Nanophyes* per stem remained significant covariates in the 'best fit' models describing both inflorescence length and number (Tables 2.6, 2.7).

At the site level, there was a strong negative association between mean *Galerucella* density and the percentage of plants that were in flower during the July visit (Figure 2.8, $F = 8.9828$, $df = 17$, $P = 0.0085$). The percentage of plants flowering at a site was positively correlated with the mean number of fruit produced per plant (Figure 2.9, $F = 4.2594$, $df = 17$, $P = 0.0556$). The number of fruit produced per gram of plant biomass was negatively associated with the densities of both herbivores (Figures 2.10a, b, *Galerucella*: $F = 7.3905$, $df = 17$, $P = 0.0152$ and *Nanophyes*: $F = 11.4106$, $df = 17$, $P = 0.0038$). Fruit density was similarly influenced by herbivore density (Figures 2.10c, d, *Galerucella*: $F = 16.6473$, $df = 17$, $P = 0.0009$, *Nanophyes*: $F = 13.3014$, $df = 17$, $P = 0.0022$,).

The independent variables were also expressed categorically as colonization history and level of present-day biological control agents. These were used to explore patterns in reproductive traits using one-way-ANOVA (Figure 2.11). The results are reflective of the linear regressions. There was no significant association between the colonization history and the number of fruit produced per gram of plant biomass (Figure 2.11a, $F = 0.8893$, $df = 2, 17$, $P = 0.1076$). When present-day *Galerucella* level is expressed as a categorical variable (i.e., absent, low, high), significantly fewer fruit are produced (per gram of plant biomass) in sites classified as having 'high' *Galerucella* density, relative to intermediate and low (Figure 2.11b, $F = 7.5429$, $df = 2, 17$, $P = 0.0054$, Tukey-Kramer's test between pairs: absent *vs*. high P = 0.0595, low *vs*. high $P = 0.0143$).

Using the mixed-modeling approach described above, I investigated the possibility of an interaction between the two biological control agents in terms of their effect on the

reproductive plant traits inflorescence number and inflorescence length. Since *Lythrum* stem height is known to be highly correlated with other plant traits (Table 2.5) it was included in the mixed-model analysis to ensure that the size of the plant was taken into consideration when evaluating the number and total length of inflorescences per plant (both of which are positively correlated with plant fruit production). In Table 2.6, three candidate models for each of the two response variables (the number of inflorescences per plant (A models) and total length of inflorescences per plant (B models)) are presented with their AICc values. From these models the 'best' were selected based on AICc. Covariates that did not improve the model were: an interaction between *Galerucella* density and stem height (models A1, B1), and an interaction between the density of the two herbivores (models A1, A2, B1, B2). In each of the two best-fit models (models A3, B3) the remaining fixed effects were stem height, *Galerucella* density (all life stages) and the *Nanophyes* density (adults) on the stem (Table 2.6). The estimates (± SE) of each of six parameters for the ($\log_{10}$) number of inflorescences per plant (model A3) and the ($\log_{10}$) total inflorescence length per plant (model B3) are presented in Table 2.7.

Species Richness

A total of 206 species of vascular plants were identified from the quadrat samples at the 18 sites. Plant orders with the most members present include the Asterales (17) and Poales (50). Occasionally, woody plants were found in the quadrats, including 13 tree species, the most common being sumac, willow, maples and dogwood. The total number of plant species per site (mean 38.17 ± 2.26), the total number of plant families per site (mean 13.33 ± 0.70), and the mean quadrat species richness (6.00 ± 0.15) are reported in Table 2.5. At the site level, I detected a negative, but non-significant association between the mean above-ground biomass of *Lythrum* and quadrat species richness ($F = 3.56$, $df = 17$, $P = 0.0778$, Figure 2.12).

No associations were detected between *Galerucella* density and total number of plant species ($F = 0.0872$, $df = 17$, $P = 0.7715$), total number of plant families ($F = 0.1886$, $df = 17$, $P = 0.6699$) or mean quadrat species richness ($F = 2.0895$, $df = 17$, $P = 0.1676$). Similarly, there was no association between colonization history and any of these measurements.

Discussion

Overall, my findings demonstrate that the present-day herbivore densities of two biological control agents, *Galerucella* and *Nanophyes*, are associated with lower reproductive success in *Lythrum* populations. However, there was no significant association between plant productivity and the density of either of the two biological control agents. Additionally, there is no evidence that the presence of the two biological control agents has had any influence on plant community species richness to date.

An expectation of classical biological control is that the released agents will establish, reproduce, increase in number and eventually spread to other host-invaded areas without assistance (Julien 1992, McFayden 1998). Furthermore, in the ideal scenario, while the population of the biological control agent is increasing, it is expected to negatively influence the fitness and performance of the targeted invasive species (e.g., through the suppression of fruiting, flowering, shoot growth and reductions in above-ground biomass) (DeClerke-Floate and Harris 2002). My findings suggest that *Lythrum* populations with a longer history with the biological control agent *Galerucella* exhibit significantly more feeding damage (Figure 2.3), supporting this anticipated effect of a release program (as in Debach and Rosen 1991, Albright 2004, Corrigan 2006, Boag and Eckert 2013).

Much of the research pertaining to the effects of *Galerucella* on *Lythrum* either pre-dates the actual releases, is from research conducted quite early in the history of the program,

or was performed in an artificial environment. One of the original motivations for a *Lythrum* biological control program was to deal with the fact that it is difficult to chemically or physically manage (Malecki and Rawinski 1985, Thompson et al. 1987, Wilcox 1989, Welling 1990, Malecki et al. 1993) and thought to outcompete native species and reduce biodiversity (Stuckey 1980, Thompson et al. 1987). Prior to the release of *Galerucella* and *Nanophyes*, both were assessed to be specialist feeders with very little probability of host-switching (Hight and Drea 1991, Kok et al. 1992, Malecki et al. 1993, Blossey et al. 1994).

Lythrum inflorescences have the potential to produce many seeds, but if the inflorescences, or individual flowers, are damaged at any stage, fewer fruits develop on that inflorescence (Katovich 2001, Van Dreische et al. 2002). Larval feeding on apical meristems by *Galerucella* (often quite early in the season), known to induce lateral growth, (Blossey and Schat 1997, Venecz and Aarssen 1998, Schat and Blossey 2005, Hunt-Joshi et. al. 2004), was clearly visible in my study populations. Not surprisingly, I detected this induced change in architecture as a positive association between *Galerucella* density and the number of lateral inflorescences. I did not, however, detect a negative association between *Galerucella* density (or exposure history) and stem height (as found by Grevstad and Hertzig 1997, Katovich 2001, Hunt-Joshi et al. 2004) or plant biomass (Quiram 2013), both of which are known to be important competitive traits in *Lythrum* (Gaudet and Keddy 1988, Agren 1996, Mal et al. 1997). My finding of an association between *Galerucella* density and a reduction in the percentage of plants flowering at a site is likely due to the feeding-associated change in inflorescence architecture, which delays the first flowering (as in Benner 1988, Venecz and Aarssen 1998). This delay can result in a reduced overall flowering period (Blossey and Schat 1997, Grevstad and Hertzig 1997), reproductive success (Rathcke and Lacey 1985) and, as

observed, the number of fruit produced (per gram of biomass) (Blossey and Schat 1997, Schat and Blossey 2005, Montague et al. 2008).

There is no existing literature on the history of *Nanophyes* in Ontario, and minimal information could be found on the effects of *Nanophyes* as a biological control agent of *Lythrum*. Additionally, because the study was not originally designed to disentangle the influences of *Galerucella* and *Nanophyes*, it was impossible to assign the various findings to one species over the other. With that in mind, my data are consistent with previous findings about the effects of *Nanophyes* on *Lythrum*. For example, the presence of *Nanophyes* is reported to be associated with premature flower drop and a reduction in seed production (Van Dreische et al. 2002), along with reductions in the viable portion of inflorescence length (St. Louis 2013). In addition to the impacts of *Galerucella*, the density of *Nanophyes* at a site was also negatively correlated with *Lythrum* fruit production (number of fruit produced per gram biomass) and fruit density.

Although feeding by *Galerucella* can cause intense defoliation (Blossey 1995), it does not often do so on the majority of the plants within a population, and tends to reduce the overall population's total leaf biomass by only about 3.5-4.5% (Dech and Nosko, 2001). Foliage-feeders can reduce a damaged plant's photosynthetic ability (Zangerl et al. 2002). New or established individual plants (native or naturalized), may take advantage of the space opened up through feeding-induced canopy reduction, eliciting a community–level response which may or may not result in an associated increase in community richness (Crawley 1989, Carson and Root 2000, Hunt-Joshi 2004).

Some amount of herbivory can be tolerated and may even be (at least temporarily) beneficial to plants (Harris 1974, Hendrix 1988, Lucas-Barbosa et al. 2013), inducing extra branching and offering the potential for increased number of inflorescences, fruit and seeds

(Benner 1988, Schat and Blossey 2005). However, defoliation thresholds are reported for many plant species, below which an individual's performance is not significantly affected due to a compensatory response (Hendrix 1988, Zehnder 1995, Kogan 1998, Pearson and Callaway 2003, Jenner et al. 2010, De Clerk-Floate 2013). My research suggests that *Lythrum* plants may be able to sustain a low level of *Galerucella* herbivory without a reduction in reproductive output or loss of biomass. However, plants at sites with an average of more than four *Galerucella* per stem produced significantly less fruit (per gram of biomass).

Plant biomass allocation may differ depending on the plant's surroundings. Being outside of its native range or simply encountering unfamiliar environmental factors can affect the way plants invest their resources (Blossey and Kamil 1996, Edwards et al. 1998, Weber and Schmid 1998, Bastolova and Kvet 2002, Stastny 2005). Although it has been suggested that in *Lythrum*, root storage takes precedence over reproduction in areas with heavy herbivory (Blossey and Schat 1997), I did not find any evidence to support this.

In my study, the adult density of the two species of biological control agents was correlated, making it difficult to assign effects on *Lythrum* to one or the other. It is possible that the impacts of the two biological control agents are mediated through different feeding strategies, and that their impacts could be additive or even synergistic. *Nanophyes* directly destroys individual flowers on inflorescences (Van Dreische et al. 2002, pers. obs.), without decreasing their length, while feeding by *Galerucella* changes plant architecture and delays the flowering (Schat and Blossey 2005). The flowers that emerge later may or may not set fruit before the end of the season due to low temperatures or pollen limitation. Thus, the change in architecture results in an increase in the number of inflorescences without necessarily increasing the overall reproductive output (supported by Katovich (2001)). Assessing the impacts and potential interactions between species can help with decision-

making around the potential release of multiple biological control agents (Louda et al. 1993, Denno et al. 1995). In my study, no interactions between the density of *Galerucella* and the density of *Nanophyes* per stem were detected in terms of the number of inflorescences or total length of inflorescences produced per plant. However, as my study was not designed in such a way that the effects of these two species could be examined independently, I cannot be certain that the effects of herbivory are due solely to one species of biological control over the other.

The application of multiple herbivores in biological control raises ecological and ethical questions about the potential for unanticipated interactive effects among the herbivores that could either reduce (Masters et al. 2001) or increase (Malecki et al. 1993) the management impact. The program to control invasive toadflax species (*Linaria vulgaris* and *L. dalmatica*), for example, included the release of multiple herbivorous agents (De Clerk-Floate and Harris 2002, McClay and De Clerk-Floate 2002) but the success of the program has been attributed to only three (De Clerck-Floate, R. and H. Cárcamo. 2011, De Clerk-Floate and Turner 2013). An important difference between these two species of *Linaria* and *Lythrum* is the number of flowering shoots that develop. *Linaria* has only one main flowering shoot, that, once damaged, fails to flower making this species well-suited for biological control, whereas *Lythrum* can produce many inflorescences even when damaged.

The implementation of a biological control program involves a great deal of research, money, time and risk management planning. Funding for these programs is mostly allotted to the first steps, with little support remaining for post-release evaluations (Ding et al. 2006). The release of biological control agents into a new environment cannot be undone and should be monitored thereafter. The challenge is to determine what indicators to record, and for how long, in order to assess the success of the program. Sometimes a reduction in the stem number, or reproductive output or biomass (Debach and Rosen 1991, Jensen et al. 2002,

Mason and Huber 2002) of the invasive plant might be used, while other times changes in biodiversity might be used to benchmark success of a biological control program (Blossey et al. 2000, Albright 2004, Grice 2004, Hulme 2006). Regardless, these assessments require repeated measures over time and a commitment to the program.

Early concerns about the potential for *Lythrum* to reduce floral and faunal biodiversity (Stuckey 1980, Thompson et al. 1987, Blossey and Skinner 2001) were highlighted by public outreach pamphlets (e.g., such as those produced in 1993 by Ducks Unlimited and The Canadian Wildlife Federation), but the actual risk of these outcomes has been debated (Anderson 1995, Treberg and Husband 1999). The negative association between *Lythrum* size (above-ground biomass) and species richness reported here supports other research indicating that the presence of *Lythrum* is associated with reduced plant diversity (Thompson et al. 1987, Blossey et al. 2001, Farnsworth and Ellis 2001, Hovick et al. 2011). However, since I have no information on the plant community composition prior to *Lythrum* invasion and subsequent *Galerucella* releases, it is not possible to draw conclusions about changes in my sites over time. Although Albright et al. (2004) reported an increase of almost five plant species/m^2 over two years as a result of increased *Galerucella* feeding, no associations were detected between higher *Galerucella* activity or length of exposure and species richness across my sites (similar to Hunt-Joshi et al. 2004). Even if herbivores successfully reduce *Lythrum*'s ability to compete, it would likely take several years before a detectable increase in plant richness is found (Carson and Root 2000, Denoth 2004).

A rebound in plant community diversity requires not only that the biological control program sufficiently reduce plant biomass/competitive ability in order to allow for new plant establishment but also that there is recruitable plant material available. Different habitat types, plant taxa and plant growth forms are variably susceptible to alien plant invasion (Doak 1992,

Lau 2006, Gaertner et al. 2009, Brody and Irwin 2012). Because of this, particular characteristics of an invasive are not necessarily intrinsically tied to biodiversity decline (Davis et al. 2011, Sax and Gaines 2003, Gaston 2010, Vila et al. 2011). Isolated sites, such as those on islands or in urban settings, may have a more difficult time recruiting native plants than those with intact ecosystems nearby. Moreover, it is more likely that species with weedy, or r-selected traits such as high seed production and wind dispersal will be recruited over those representative of the 'pre-invaded' community (Lawton and Brown 1986, Sax and Brown 2000). Indeed, it is often found that one invasive plant species is simply competing with another (Huffaker and Kennett 1959). For example, at my study site, Jebb's Creek, where a swathe of *Lythrum* was mowed over the course of the summer, wild parsnip (*Pastinaca sativa*, an invasive and toxic plant) replaced it as the dominant species. Interestingly, the introduction of exotic species may act to increase species richness (Treberg and Husband 1999, Sax and Gaines 2003). Thus, it is perhaps not too surprising that it is difficult to make strong conclusions about the influence of a biological control release on species richness at my sites.

Assessing a site's history with the two species of biological control was challenging for a number of reasons. First, the number of *Galerucella* individuals released at different sites over the years was not consistent, nor were the details of a release consistently recorded (i.e., numbers released, exact locations, information on re-distribution or established populations). Second, no information is available about the history, presence and impact of *Nanophyes* in Ontario over the last ten years due to lack of monitoring (i.e., especially after Corrigan 2006). Last, local adaptation to herbivores and other environmental conditions could influence the evolution of ecotypes that may in turn influence the relationships between herbivory and plant traits (Ehler et al. 2004, Müller-Schärer et. al 2004). If present, this type

of adaptation could make it difficult to compare sites in terms of the influences of herbivory.

Optimally, biological control programs should continue the monitoring process over the long term, even in cases where a program has been deemed a success, because of the potential for changes in the biology of the system over time (Mason and Gillespie 2013). In addition, it is important that population information for the target plant species and the surrounding plant community be monitored and reported, in addition to the data on insect feeding that is typically collected. Several Canadian biological control programs, commonly referred to as successes based on the establishment and spread of the agents, were reviewed by Mason and Huber in 2002; for example, the control of *Centaurea diffusa* (diffuse knapweed) (Bourchier et al. 2002a), *Euphorbia spp.* (leafy and Cypress spurge) (Bourchier et al. 2002b), *Linaria spp.* (Yellow and Dalmatian toadflax) (DeClerke-Floate and Harris 2002, McClay and De Clerk-Floate 2002), *Hypericum perforatum* (St. John's wort, Klamath weed) (Jensen et al. 2002) as well as *Lythrum* (Lindgren et al. 2002). A decade later, an additional update to these same programs was delivered by Mason and Gillespie (2013), they reported that for several programs previously found to be thriving, the success either took longer than expected to develop, was patchy, or involved agents that were slow to establish outside the release area or were limited in their ability to control a quickly spreading target host species (Bourchier and Van Hezewijk 2013a, Bourchier and Van Hezewijk 2013b). There were also concerns about the actual taxonomic identity of some of the original releases (i.e., taxonomic error was recently revealed by more modern genetic analysis) and the need in some cases for biological control agent supplementation (De Clerk-Floate and Turner 2013, De Clerk-Floate and McClay 2013). Mason and Gillespie (2013) include recommendations for long-term monitoring and further improvement of biological control programs based on an increased

understanding of the genetic diversity of both the host species and introduced biological control agents.

Without continued monitoring of biological control programs there is the real potential to miss important developments and possibly exaggerate or fail to accurately assess the magnitude of the success or the plant responses (Agrawal and Kotanen 2003). To determine whether the Ontario *Lythrum* biological control program actually reduced sexual reproduction and stem density and/or lead to an increase in local plant biodiversity, a long term monitoring program is recommended.

Conclusions

The results of my field study indicate that the present day density of two biological control agents, *Galerucella* and *Nanophyes*, directly affects *Lythrum* reproductive output; this is consistent with reports dealing with other sites or laboratory trials (Blossey and Schat 1997, Katovich et al. 2001, Van Driesche et al. 2002, Landis et al. 2003, Schat and Blossey 2005). This information should be considered when planning future releases of biological control agents to the system (as in Jenner et al. 2010).

Although the impacts of the two herbivores detected in this study may have contributed to a reduction in *Lythrum's* competitive advantage by reducing the fruit produced and thus seeds that enter the local seed bank (or disperse to new areas), this may not necessarily translate into a reduction in population size. Since a single *Lythrum* plant has the capacity to produce such a large number of seeds in a single season, a reduction in flowering in a proportion of the population may not work to manage populations or assist in the recovery of the native plant community. It has been suggested that extensive, continuous feeding damage over a period of time is required in order to expect a decline in year-to-year

recruitment (Powell 1988, Blossey and Schat 1997, Strauss 1991, Karban and Strauss 1993, Denoth and Myers 2004).

While my study was originally designed to understand the impacts of *Galerucella*, the discovery that many of my sites were colonized by a second biological control agent (*Nanophyes*), offered the opportunity to explore the impacts of two introduced herbivores on their intended host species and the surrounding plant community (as in Strauss and Irwin 2004, Poveda et al. 2005, Swope and Parker 2010, Swope and Parker 2012). However, as the adult site density of the two biological control agents was positively correlated, it was not possible to assess the impacts of either herbivore separately. Future research efforts could, for example, intentionally exclude or introduce one of the biological control agents, either at the site level or within a site, in order to experimentally manipulate their individual impacts. Otherwise, a longer-term study would allow a researcher to assess the separate herbivore densities in advance and make site selections based in this information. Future studies should also collect more information on site conditions (e.g., soil nutrients, soil moisture, aspect, light conditions, etc.) in order to better understand, and possibly control for, the influence of site variability in these factors.

References

Agrawal A. A.and P. M. Kotanen. 2003. Herbivores and the success of exotic plants: a phylogenetically controlled experiment. Ecology Letters **6**:712-715.

Albright M. F, W. N. Harman, S. S. Fickbohm, H. Meehan, S. Groff, and T. Austin. 2004. Recovery of native flora and behavioural responses of *Galerucella sp*. following biocontrol of purple loosestrife. The American Midland Natualist. **152**:248-254.

Ali, S. and C. Verbeek. 1999. The Alberta purple loosestrife eradication programs 1999 Status Report. Alberta Agriculture, Food and Rural Devevopment, Edmonton Alberta.

Anderson D. R., W. A. Link, D. H. Johnson and K. P. Burnham. 2001. Suggestions for presenting the results of data analysis. Journal of Wildlife Management **65**:373-378.

Anderson, M. G. 1995. Interactions between *Lythrum salicaria* and native organisms: A critical review. Environmental Management **19**:225-231.

Argen J. 1996. Population size, pollinator limitation ad seed set in the self-incompatible herb *Lythrum salicaria.* Ecological Society of America **77**:1779-1790.

Barber, N. A., L. S. Adler, and H. L. Bernardo. 2011. Effects of above- and belowground herbivory on growth, pollination, and reproduction in cucumber. Oecologia **165**:377-386.

Barber, N. A., L. S. Adler, N. Theis, R. V. Hazzard, and E. T. Kiers. 2012. Herbivory reduces plant interactions with above- and belowground antagonists and mutualists. Ecology **93**: 1560-1570.

Barrett, S. C. H. and L. D. Harder. 1996. Ecology and evolution of plant mating. Trends in Ecology and Evolution **11**:73-79.

Barrett, S. C. H., L. D. Harder, and W. W. Cole. 1994. Effects of flower number and position on self-fertilization in experimental populations of *Eichhornia paniculata* (Pontederiaceae). Functional Ecology **8**:526-535.

Bastolová D. and J. Kvet 2002. Differences in dry weight partitioning and flowering phenology between native and non-native plants of purple loosestrife (*Lythrum salicaria* L.). Flora **197**:332-340.

Batra, S.W.T. , D. Schroder and P.E. Boldt and W. Mendl.1986. Insects associated with purple loosestrife (*Lythrum salicaria* L.) in Europe. Proceedings of the Entomological Society of Washington **88**:748-759.

Belsky, A. J., W. P. Carson, C. L. Jensen, and G. A. Fox. 1993. Overcompensation by plants: herbivore optimization or red herring? Evolutionary Ecology **7**:109–121.

Benner, B. L. 1988. Effects of apex removal and nutrient supplementation on branching and

seed production in *Thlaspi arvese* (Brassicaceae). American Journal of Botany. **75**:645-651.

Bergelson, J and M.J. Crawley 1982. The effects of grazers on the performance of individuals and populations of Scarlet-gilia, *Ipomopsis aggregata*. Oecologia **90**:435- 444.

Blossey, B. 1995a. A comparison of various approaches for evaluating potential biological control agents using insects on *Lythrum salicaria*. Biological Control **5**:113-122.

Blossey, B. 1995b. Coexistence of two leaf-beetles in the same fundamental niche. Distribution, adult phenology and oviposition. Oikos **74**:225-234.

Blossey, B. and J. Kamil. 1996. What determines the increased competitive ability of invasive non-indigenous plants? Pages 3–9 *in* V. C. Moran and J. H. Hoffman, editors. Proceedings of the IX International Symposium on Biological Control of Weeds, University of Cape Town, Stellenbosh, South Africa.

Blossey, B. and M. Schat. 1997. Performance of *Galerucella calmariensis* (Coleoptera: Chrysomelidae) on different North American populations of purple loosestrife. Environmental Entomology **26**:439-445.

Blossey, B., D. Schroeder, S. D. Hight, and R. A. Malecki. 1994. Host specificity and environmental impact of two leaf beetles (*Galerucella calmariensis* and *G. pusilla*) for biological control of purple loosestrife (*Lythrum salicaria*). Weed Science **42**:134-140.

Blossey, B., L. C. Skinner, and J. Taylor. 2001. Impact and management of purple loosestrife (*Lythrum salicaria*) in North America. Biodiversity and Conservation **10**:1787-1807.

Boag A.E. and C. G. Eckert. 2013. The effect on host abundance on the distribution and impact of biocontrol agents on purple loosestrife. Bioscience **20**:90-99.

Bourchier, R.S., K. Mortenson and M. Crowe. 2002. *Centaurea diffusa* Lamarck, Diffuse Knapweed and *Centaurea maculosa* Lamarck, Spotted Knapweed (Asteraceae). Pages 302-314 *in* P. G. Mason and J. T. Huber, editors. Biological Control Programmes in Canada, 1981-2000. CABI Publishing, Wallingford, UK

Bourchier R.S., S. Erb, A.S. McClay and A. Gassman. 2002. *Euphorbia esula* (L.), Leafy spurge and *Euphorbia cyparissias* (L.) Cypress sprurge. (Euphorbiacea) Pages 346-358 *in* P. G. Mason and J. T. Huber, editors. Biological Control Programmes in Canada, 1981-2000. CABI Publishing, Wallingford, UK

Bourchier R.S. and B.H Van Hezwijk. 2013a. Pages 302-306 *in* P.G. Mason and D.R. Gillespie editors. Biological Control Programmes in Canada, 2001-2012. CABI Publishing, Wallingford, UK.

Bourchier R.S.,and B.H Van Hezwijk. 2013b. *Euphorbia esula* (L.), Leafy spurge. Pages 315-320 *in* P.G. Mason and D.R. Gillespie editors. Biological Control Programmes in Canada, 2001-2012. CABI Publishing, Wallingford, UK.

Brody, A. K. 1997. Pollinators, herbivores and seed predators on flowering phenology. Ecology. **78**: 1624-1631.

Brody, A. K. and R. E. Irwin. 2012. When resources don't rescue: Flowering phenology and species interactions affect compensation to herbivory in *Ipomopsis aggregata*. Oikos **121**:1424-1434.

Carson, W.P. and R.B. Root. 2000. Herbivory and plant species coexistence: community regulation by an outbreaking phytophagous insect. Ecological Monographs **70**:73–99.

Colautti R. and S. Barrett. 2011. Population divergence along lines of genetic variance and covariance in the invasive plant *Lythrum salicaria* in Eastern North America. Evolution **65**:2514-29.

Conner, J. K. and S. Rush. 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. Oecologia **105**:509-516.

Corrigan, Jim. 2006. University of Guelph researched and then produced a document entitled 'Ten Years After' – A Summary of Results from the Ontario Purple Loosestrife Biological Control Programs for the Ontario Ministry of Natural Resources (OMNR). (unpublished)

Corrigan J., D.R. Gilespie, R. De Clerk-Floate and P. G. Mason. 2013. *Lythrum salicaria* L., Purple Loosestrife (Lythraceae). Pages 363-366 *in* P. G. Mason and J. T. Huber, editors. Biological Control Programmes in Canada, 981-2000. CABI Publishing, Wallingford, UK.

Crawley, M.J. 1989. Insect herbivores and plant population dynamics. Annual Review of Entomology. **34**:531-564.

Davies K. F., P. Chesson, S. Harrison, B. D. Inouye, B. A. Melbourne and K. J. Rice. 2005. Spatial heterogeneity explains the scale dependence of the native-exotic diversity relationship. Ecology **86**:1602-10.

Davis, M. A. 2003. Biotic globalization: Does competition from introduced species threaten biodiversity? Bioscience **53**:481-489.

Davis M. A. M. K. Chew, R. J. Hobbs, A.E. Lugo, J. J. Ewel, G. J. Vermeij, J. H. Brown, M. L. Rosenweig, M. R. Gardner, S. P. Carroll, K. Thompson, S. T. A. Pickett, J. C. Stromberg, P. Del Tredici, K. N. Sudnig, J. G. Ehrenfeld, J. P. Grime, J. Mascaro, J. C. Briggs. 2011. Don't judge a species by its origins. Nature **474**:153-154.

De Clerck-Floate, R. and H. Cárcamo. 2011. Biocontrol arthropods: new denizens of Canada's grassland agroecosystems. Pages 291–321 *in* K. Floate, K. editor. Arthropods of Canadian Grasslands, Vol. 2: Inhabitants of a Changing Landscape. Biological Survey of Canada Monograph Series No. 4, Biological Survey of Canada, Ottawa, Ontario, Canada.

De Clerk-Floate and Harris 2002. *Linaria dalmatica* (L) Miller, Dalmation toadflax. (Scrophulariaceae). Pages 368-375 *in* P. G. Mason and J. T. Huber, editors. Biological Control Programmes in Canada, 1981-2000. CABI Publishing, Wallingford, UK.

De Clerk-Floate R. and A. S. McClay. 2013. *Linaria vulgaris* Mill., Yellow toadflax (Plantaginaceae), Pages 354-362 *in* P.G. Mason and D.R. Gillespie editors. Biological Control Programmes in Canada, 2001-2012. CABI Publishing, Wallingford, UK.

De Clerk-Floate R. and S. C. Turner. 2013. *Linaria dalmatica* (L) Miller, Dalmation toadflax (Plantaginaceae). Pages 342-353 *in* P.G. Mason and D.R. Gillespie editors. Biological Control Programmes in Canada, 2001-2012. CABI Publishing, Wallingford, UK.

DeBach, P. and D. Rosen. 1991. Biological control by natural enemies, 2nd edition. Cambridge University Press. Cambridge, UK.

Denoth, M. and J.H. Myers. 2005. Variable success of biological control of *Lythrum salicaria* in British Columbia. Biological Control **32**:269-279.

Ding, J. B. Blossey, Y. Du and F. Zheng. 2006. Impact of *Galerucella birmanica* (Coleoptera: Chrysomelidae) on growth and seed production of *Trapa natans*. Biological Control **37**:338–345.

Doak, D. F. 1992. Lifetime impacts of herbivory for a perennial plant. Ecology **73**:2086-2099.

Douglas, H., P. Bouchard, R.S. Anderson, P. de Tonnancour, R. Vigneault and R. P. Webster. 2013. New Curculionoidea (Coleoptera) records for Canada. Zookeys **309**:13-48.

Eckert, C. G. and S. C. H. Barrett. 1992. Stochastic loss of style morphs from populations of tristylous *Lythrum salicaria* and *Decodon verticillatus* (Lythraceae). Evolution **46**:1014-1029.

Edwards, K. R, J. Kvet and M.S. Adams. 1998. Comparison of the demographics of native and invasive populations of *Lythrum salicaria* L. Applied Vegetative Science **1:** 267–280.

Ehler, L.E. 2000. Critical issues related to nontarget effects in classical biological control of insects. Pages 3-3 *in* P. A. Follett and J. J. Duan, editors. Nontarget Effects of Biological Control. Kluwer Academic Publishers, Norwell, Massachusetts, USA.

Ehler, L. E., R. Sforza, and T. Mateille. 2004. Genetics, evolution, and biological control. CABI, Wallingford,UK.

Farnsworth, E. and D. Ellis. 2001. Is purple loosestrife (*Lythrum salicaria*) an invasive threat to freshwater wetlands? Conflicting evidence from several ecological metrics. Wetlands **21**:199-209.

Ferrarese E. R. and R. J. Garono. 2010. Purple loosestrife biocontrol agent aggregation and overwintering are influenced by tidal inundation. Wetlands **31**:113-123.

Gaertner, M., A. Den Breeyen, C. Hui and D.M. Richardson. 2009. Impacts of alien plant invasions on species richness in Mediterranean-type ecosystems: a meta-analysis. Progress in Physical Geography. **33**:319-338.

Gaston, K. J. 2010. Valuing common species. Science **327**:154-155.

Gordon, D.R., 1998. Effects of invasive, non-indigenous plant species on ecosystem process: lessons from Florida. Ecological Applications **8:**975-989.

Grevstad F.S. and A. L. Hertzig 1997. Quantifying the effects of distance and conspecifics on colonization: experiments and models using the loosestrife beetle, *Galerucella calmeriensis.* Oecologia **110**:60-68.

Grevstad. F.S. 2006. An experimental test of the effect of initial colony size on the probability of population establishment. Bulletin of the Ecological Society of America. **77**:172-172.

Grice, A. C. 2004. Weeds and the monitoring of biodiversity in Australian rangelands. Austral Ecology. **29**:51–58.

Gronemeyer, P. A., B. J. Dilger, J. L. Bouzat, and K. N. Paige. 1997. The effects of herbivory on paternal fitness in scarlet gilia: Better moms also make better pops. The American Naturalist **150**:592-602.

Gurevitch, J. and D.K. Padilla. 2004. Are invasive species a major cause of extinctions? Trends Ecology and Evolution. **19**:470–474.

Hambäck, P. A., 2001. Direct and indirect effects of herbivory: Feeding by spittlebugs affects pollinator visitation rates and seed set of *Rudbeckia hirta*. Ecoscience **8**:45-50.

Harris, P. 1974. A possible explanation of plant yield increases following insect damage. Agro-Ecosystems **1**:219-225.

Hendrix S. D. 1988. Herbivory and its impact on plant reproduction. Pages 246–263 *in* J. Lovett-Doust, L. Lovett-Doust, editors. Plant Reproductive Ecology: Patterns and Strategies. Oxford University Press, New York.

Hight S. and J. J. Drea. 1991. Prospects for a classical control project against purple loosestrife, Lythrum salicaria L. Natural Areas Journal **11**: 151-157.

Hovick, S. M., D. E. Bunker, C. J. Peterson, and W. P. Carson. 2011. Purple loosestrife suppresses plant species colonization far more than broad-leaved cattail: Experimental evidence with plant community implications. Journal of Ecology **99**:225-234.

Hladun, K. R. and L. S. Adler. 2009. Influence of leaf herbivory, root herbivory, and pollination on plant performance in *Cucurbita moschata*. Ecological Entomology **34**:144-152.

Huffaker, C.B. and C.E. Kennett. 1959. A ten-year study of the vegetational changes associated with biological control of Klamath weed. Journal of Range Management **12**:69-82.

Hulme, P.E. 2006. Beyond control: wider implications for the management of biological invasions. Journal Applied Ecology. **43**:835–847.

Hunt-Joshi, T. R and B. Blossey. 2005. Interactions of root and leaf herbivores on purple loosestrife (*Lythrum salicaria*). Oecologia. **142**:554-563.

Hunt-Joshi, T. R. and B. Blossey, and R. B. Root. 2004. Root and leaf herbivory on *Lythrum salicaria*: Implications for plant performance and communities. Ecological Applications **14**:1574-1589.

Jenner, W. H., P.G. Mason, N. Cappuccino and U. Kuhlmann. 2010. Native range assessment of classical biological control agents: impact of inundative releases as pre-introduction evaluation. Bulletin of Entomological Research **100**:387-394.

Jensen K. I. N., P. Harris and M. G Sampsom. 2002. *Hypericum perforatum* L. St. John's Wort (Cluiasceae). Pages 361-368 *in* P. G. Mason and J. T. Huber, editors. Biological Control Programmes in Canada, 1981-2000. CABI Publishing, Wallingford, UK.

JMP, version 10. SAS Institute Inc., Cary, NC.1998-2011.

jmp.com/support/help/Mixed_and_Random_Effect_Model_Reports_and_Options.shtml

Jogesh, T., D. Carpenter and N. Cappuccino. 2008. Herbivory on invasive exotic plants and their non-invasive relatives. Biological Invasions **10**:797-804.

Jones E. and R. Gomulkiewicz. 2012. Biotic interactions, rapid evolution and the establishment of introduced species. The American Naturalist **179**:E28-E36.

Julien M. H. 1992. Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds. 3rd ed. Wallingford, Oxford: CAB Interantional.186 pp.

Karban, R. and S. Y. Strauss. 1993. Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. Ecology **74**:39-46.

Katovich, E. J. S., D. W. Ragsdale, L. C. Skinner, and R. L. Becker. 2001. Effect of *Galerucella* spp. feeding on seed production in purple loosestrife. Weed Science **49**:190-194.

Kogan, M.1998. Integrated pest management: Historical perspectives and contemporary developments. Annual Review of Entomology **43**:243-270.

Kok, L. T., T. J. McAvoy, R. A. Malecki, S. D. Hight, J. J. Drea, and J. R. Coulson. 1992. Host specificity tests of *Galerucella calmariensis* (L.) and *G. pusilla* (Duft.) (Coleoptera: Chrysomelidae), potential biological control agents of purple loosestrife, *Lythrum salicaria* L. (Lythraceae). Biological Control **2**:282-290.

Krupnick, G. A. and A. E. Weis. 1999. The effect of floral herbivory on male and female reproductive success in *Isomeris arborea*. Ecology **80**:135-149.

Krupnick, G. A., A. E. Weis, and D. R. Campbell. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. Ecology **80**:125-134.

Lande R. and S. J. Arnold.1983. The measurement of selection on correlated characters. Evolution **76**:1210-1226.

Landis, D. A., D. C. Sebolt, M. J. Haas, and M. Klepinger. 2003. Establishment and impact of *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae) on *Lythrum salicaria* L. and associated plant communities in Michigan. Biological Control **28**:78-91.

Lau J. A. 2006. Evolutionary responses of naïve plants to novel community members. Evolution **60:**56-63.

Lehtilä, K. and S. Y. Strauss. 1997. Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. Oecologia **111**:396-403.

Lehtilä, K. and S. Y. Strauss. 1999. Effects of foliar herbivory on male and female reproductive traits of wild radish, *Raphanus raphanistrum*. Ecology **80**:116-124.

Lennartsson, T., J. Tuomi, and P. Nilsson. 1997. Evidence for an evolutionary history of overcompensation in the grassland biennial *Gentianella campestris* (Gentianaceae). American Naturalist **149:**1147–1155.

Levine J. M., M. Vila, C. M. D. Antonio, J. S. Dukes, K. Grigulis and S. Lavorel. 2003. Mechanisms underlying the impacts of exotic plant invasions. Proceedings of the Royal Society B. **1517:**775-781.

Lindgren, C. J. 1999. Performance of a biological control agent, *Galerucella calmariensis* L. (Coleoptera: Chrysomelidae) on purple loosestrife *Lythrum salicaria* L. in Southern Manitoba (1993-1998). Pages 367-382 *in* Proceedings of the X International Symposium on Biological Control of Weeds, Montana State University, Bozeman, Montana, USA.

Lindgren, C. J., J. Corrigan, and R. A. De Clerck-Floate. 2002. *Lythrum salicaria* L., purple loosestrife (Lythraceae). Pages 383-390 *in* P. G. Mason and J. T. Huber, editors. Biological Control Programmes in Canada, 1981-2000. CABI Publishing, Wallingford, UK.

Louda, S. M., K. H. Keeler, and R. D. Holt. 1990. Herbivore influences on plant performance

and competitive interactions. Pages 413-444 *in* J. B. Grace and D. Tilman, editors. Perspectives on plant competition. Academic, New York.

Louda, S. M., R. W. Pemberton, M. T. Johnson and P. A. Follett, P.A. 2003. Nontarget effects- the Achilles' Heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. Annual Review of Entomology **48**:365-396.

Lucas-Barbosa, D., J. J. A. van Loon, R. Gols, T.A. van Beek, M. Dicke. 2013. Reproductive escape: annual plant responds to butterfly eggs by accelerating seed production. Functional Ecology **27:**245–254.

Mack, R. N., D. Simberloff, W. M. Londsdale, H. Evans, M. Clout and F. A. Bazzaz. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. Ecological Applications **10**: 689-710.

Mal, T. K., J. Lovett-Doust, and L. Lovett-Doust. 1997. Time-dependent competitive displacement of *Typha angustifolia* by *Lythrum salicaria*. Oikos **79**:26-33.

Malecki, R. A., B. Blossey, S. D. Hight, D. Schroeder, L. T. Kok, and J. R. Coulson. 1993. Biological control of purple loosestrife. BioScience **43**:680-686.

Mason P. G. and J. T. Huber, editors. Biological Control Programmes in Canada, 1981-2000. CABI Publishing, Wallingford, UK.

Mason P. G., J. T. Kabaluk, B. Spencer and D. R. Gillespie. 2013. Pages 1-5 *in* P.G. Mason and D.R. Gillespie editors. Biological Control Programmes in Canada, 2001-2012. CABI Publishing, Wallingford, UK.

Masters G. J., T. Hefin Jones and M. Rogers. 2001. Host-plant mediated effects of root herbivory on insect seed predators and their parasites. Oecologia. **127**:246-20.

McClay A. S. and R. De Clerk-Floate 2002. *Linaria vulgaris* (L.) Miller (Scrophulariaceae). Pages 375-382 *in* P. G. Mason and J. T. Huber, editors. Biological Control Programmes in Canada, 1981-2000. CABI Publishing, Wallingford, UK.

McFayden, R. E. C.1998. Biological control of weeds. Annual Review of Entomology. **43**:369-393.

McNaughton, S. J. 1983. Compensatory plant growth as a response to herbivory. Oikos **40**:329-336.

Melbourne B. A., H. V. Cornell, K. F. Davies, C. J. Dugaw, S. Elmendorf, A. L. Freestone R. J. Hall, S. Harrison, A. Hastings, M. Holland, M. Holyoak, J. Lambrinos, K. Moore, H. Yokomizo. 2007. Invasion in a heterogeneous world: resistance, coexistence or hostile takeover? Ecology Letters **10**: 77-94.

Messing, R. H. 2000. The impact of nontarget concerns on the practice of biological control. Pages 45–55 *in* P.A. Follett and J.J. Duan editors. Nontarget Effects of Biological

Control. Kluwer Academic Publishers, Norwell, Massachusetts, USA.

Montague, J. L., S. C. H Barrett and C. G. Eckert. 2008. Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythrum salicaria*, Lythraceae). Journal of Evolutionary Biology. **21**: 234-245.

Mothershead, K. and R. J. Marquis. 2000. Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in *Oenothera macrocarpa*. Ecology **81**:30-40.

Müller-Schärer, H. U. Schaffner and T. Steinger. 2004. Evolution in invasive plants: implications for biological control. Trends in Ecology and Evolution. **19**:417-422

Mutikainen, P. and L. F. Delph. 1996. Effects of herbivory on male reproductive success in plants. Oikos **75**:353-358.

Myers, J. H. 2008. *In* Proceedings of the XII International Symposium on Biological Control of Weeds. M. H. Julien, R. Sforza, M. C. Bon, H. C. Evans, P.E. Hatcher, H. L. Hinz, and B. G. Rector, B. G. editors. CAB International Wallingford, UK.

Myers, J. H. and D. R. Bazely. 2003. Ecology and Control of Introduced Plants. Cambridge University Press, Cambridge, UK.

Myers, J. H. and M. Denoth. 1999. Endangered Species Recovery Fund Report, 31 November, 1999. University of British Columbia, Vancouver BC.

Myers, J. H., C. Jackson, H. Quinn, S.R. White and J.S. Cory. 2009. Successful biological control of diffuse knapweed, *Centaurea diffusa*, in British Columbia, Canada. Biological Control **50**:66-72.

Myers, J. H. and C. Risley. 2000. Why reduced seed production is not necessarily translated into successful biological weed control. Pages 569–581 *in* N. Spencer, editor. Proceedings X. International Symposium Biological Control of Weeds. Montana State University, Bozeman, MO.

Newcomb, L. 1977. Newcomb's Wildflower Guide. Time Warner Book Group. New York, USA.

Niesenbaum, R. A. 1996. Linking herbivory and pollination: Defoliation and selective fruit abortion in *Lindera benzoin*. Ecology **77**:2324-2331.

Norman K., N. Cappuccino and M .R. Forbes. 2009. Parasitism of a successful weed biocontrol agent, *Neogalerucella calmeriensis*. The Canadian Entomologist. **141**:609-613.

Nötzold, R., B. Blossey, and E. Newton. 1998. The influence of below ground herbivory and plant competition on growth and biomass allocation of purple loosestrife. Oecologia **113**:82-93.

Ohashi, K. and T. Yahara. 1998. Effects of variation in flower number on pollinator visits in *Cirsium purpuratum* (Asteraceae). American Journal of Botany **85**:219-224.

O'Neil, P. 1997. Natural selection on genetically correlated phonological characters in *Lythrum salicaria* (Lythraceae). Evolution. **51**:267-274.

Paige, K. N. and T. G. Whitham. 1987. Overcompensation in response to mammalian herbivory - the advantage of being eaten. American Naturalist **129**:407-416.

Paige, K. N. 1996. Herbivory and *Ipomopsis aggregata*: Differences in response, differences in experimental protocol: a reply to Bergelson and Crawley. The American Naturalist. **143:**739-749.

Pearson, D. E. and R. M. allaway 2003. Indirect effects of host-specific biological control agents. Trends in Ecology and Evolution **18**:456-461.

Pellegrino, G. and A. Musacchio. 2006. Effects of defoliation on reproductive success in two orchids, *Serapias vomeracea* and *Dactylorhiza sambucina*. Annales Botanici Fennici **43**:123-128.

Peterson, R. T. and M. McKenny. 1996. Peterson's Field Guides, Wildflowers Northeastern/ North-central North America. Houghton Mifflin Company, New York, USA.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics **52**:273-288.

Piper G.L., E.M. Coombs, B. Blossey, P.B. McEvoy and S.S. Schooler. 2004. Purple loosestrife. Pages 281–292 *in* E.M. Coombs, J.K. Clark, G.L. Piper, A.F. Cofrancesco, editors. Biological control of invasive plants in the United States. Oregon State University Press, Corvallis.

Powell R.D. 1988. Diffuse Knapweed (*Centaurea diffusa*): Reproductive thresholds, Population ecology and responses to the introduction on the buprestid beetle *Sphhenoptera jugoslavica*. Doctoral Thesis, University of British Columbia. .

Powell K. I., J. M. Chase and T. M Knight. 2013. Invasive plants have scale dependant effects on diversity by altering species-area relationships. Science **339**:316-318.

Poveda, K., I. Steffan-Dewenter, S. Scheu, and T. Tscharntke. 2003. Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set. Oecologia **135**:601-605.

Quinn, G. P. and M. J. Keough. 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

Rathcke, B. and E. P. Lacey. 1985. Phenological patterns of terrestrial plants. Annual Review

Ecology, Evolution and Systematics. **16**:179-214.

Rausher, M. D. and P. Feeny. 1980. Herbivory, plant density, and plant reproductive success: The effect of *Battus philenor* on *Aristolochia reticulata*. Ecology **61**: 905-917.

Rush, S., J. K. Conner, and P. Jennetten. 1995. The effects of natural variation in pollinator visitation on rates of pollen removal in wild radish, *Raphanus raphanistrum* (Brassicaceae). American Journal of Botany **82**:1522-1526.

Russell-Mercier, J. 2013. A study of the impact of an introduced herbivore on pollinator-mediated interactions and female fitness in *Lythrum salicaria.* Masters Thesis, University of Ottawa.

Schat, M. and B. Blossey. 2005. Influence of natural and simulated leaf beetle herbivory on biomass allocation and plant architecture of purple loosestrife (*Lythrum salicaria* L.). Environmental Entomology **34**:906-914.

Sharaf, K. E. and M. V. Price. 2004. Does pollination limit tolerance to browsing in *Ipomopsis aggregata*? Oecologia **138**:396-404.

Simons, A. and M. O. Johnston. 1999. The Cost of Compensation. American Naturalist **153**:683-687.

Simberloff, D. 2011. Risks of biological control for conservation purposes. BioControl 57: 263–276

Skinner, L. 1996. Integrated control of Purple loosestrife in Minnesota. Minnesota Department of Natural Resources. Fifteenth North American Prairie Conference. 219-222.

Sax D. F. and J. H. Brown. 2000. The paradox of invasion. Global Ecology and Biogeography **9**:363-371.

Sax, D. F. and S. D. Gaines. 2003. Species diversity: from global decreases to local increases. Trends Ecology and Evolution **19**:470–474.

Schooler, S. S. and P. B. McEvoy. 2006. Relationship between insect density and plant damage for the golden loosestrife beetle, *Galerucella pusilla*, on purple loosestrife (*Lythrum salicaria*). Biological Control **36**:100-105

Stephenson, A. G. 1981. Flower and fruit abortion: Proximate causes and ultimate functions. Annual Review of Ecology and Systematics **12**:253-279.

St. Louis, E. 2013. New Ontario records for *Nanophyes m. marmoratus* (Goeze 1777) (Coleoptera: Brentidae) introduced into North America for the classical biological control of purple loosestrife. Journal of the Entomological Society of Ontario. **144**: 19-123.

Strauss, S. Y. 1991. Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. Ecology **72**:543-558.

Strauss S. Y. and A. A. Agrawal 1999. The ecology and evolution of plant tolerance to herbivory. Trends Ecology and Evolution 14:179-185.

Strauss, S. Y., J. K. Conner, and K. P. Lehtila. 2001. Effects of foliar herbivory by insects on the fitness of *Raphanus raphanistrum*: Damage can increase male fitness. The American Naturalist **158**:496-504.

Strauss, S. Y. and R. E. Irwin. 2004. Ecological and evolutionary consequences of multispecies plant-animal interactions. Annual Review of Ecology, Evolution, and Systematics **35**:435-466.

Strauss, S. Y. J. A. Lau and S. P. Carol. 2006. Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? Ecology Letters **9**:357-374.

Strauss, S. Y., C. O. Webb and N. Salamin. 2006. Taxa less related to native species are more invasive. Proceedings of the National Academy of Sciences of the United States of America **103**:5841-5845.

Stastny M., U. Schaffner and E. Elle. 2005. Do vigour of introduced populations and escape form specialist herbivores contribute to invasiveness? Journal of Ecology **93**:27-37.

Stuckey R. L.1980. Distributional history of *Lythrum salicaria* (purple loosestrife) in North America. Bartonia **47**:3-20.

Suárez, L. H., W. L. Gonzalez, and E. Gianoli. 2009. Foliar damage modifies floral attractiveness to pollinators in *Alstroemeria exerens*. Evolutionary Ecology **23**:545-555.

Swope, S. M. and I. M. Parker. 2012. Complex interactions among biological control agents, pollinators, and an invasive weed: A structural equation modeling approach. Ecological Applications **22**:2122-2134.

Syrett, P. D., T. Briese and J. H. Hoffmann. 2000. Success in biological control of terrestrial weeds by arthropods. Pages 189–230 *in* G.M. Gurr and S.D. Wratten, editors.Biological Control: Measures of Success. Kluwer Academic Publishers. New Zealand.

Thompson, D. W., R. L. Stuckey, and E. B. Thompson. 1987. Spread, impact and control of purple loosestrife (*Lythrum salicaria*) in North American wetlands. Fish and Wildlife Research 2. U.S. Department of the Interior, Fish and Wildlife Service, Washington D.C.

Thomas M.B. and A.M. Reid. 2007. Are exotic natural enemies an effective way of controlling invasive plants? Trends in Ecology and Evolution. **22**:447-453.

Treberg, M. A. and B. C. Husband. 1999. Relationship between the abundance of *Lythrum salicaria* (purple loosestrife) and plant species richness along the Bar River, Canada. Wetlands. **19**:118-125.

Turcotte M. M., T. J. Davies, C. J. M. Thomsen and M. T. J. Johnson. 2014. Macroecological and macroevolutionary patterns of leaf herbivory across vascular plants. Proceedings of the Royal Society B. **281**:20140555.

Van Driesche, R., B. Blossey, M. Hoddle, S. Lyon and R. Reardon. 2002. Biological control of invasive plants in the Eastern United States. USDA Forest Service FHTET-2002-04. 413 pp.

van Lenteren J. C., J. Bale, F. Bigler. H. M. T. Hokkanen and A. J. M. Loomans. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. Annual Review of Entomology. **51**:609-634.

Vázquez, D. P. and D. Simberloff. 2004. Indirect effects of an introduced ungulate on pollination and plant reproduction. Ecological Monographs **74**:281-308.

Vila, M., J. L. Espinar, M. Hejda, P. E. Hulme, V. Jaros, J. L. Maron, J. Pergl, U. Schaffner, Y. Sun and P. Pysek. 2011. Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecology Letters **14:**702-708.

Venecz, J. I. and L. W. Aarssen. 1998. Effects of shoot apex removal in *Lythrum salicaria* (Lythraceae): Assessing the costs of reproduction and apical dominance. Annales Botanici Fennici **35:**101-111.

Weber, E. and B. Schmid. 1998. Latitudinal populations differentiation in two species of *Solidago* (Asteraceae) introduced into Europe. American Journal of Botany. **85:** 110–121.

Welling C. H., R. L. Becker. 1990. Seed-bank dynamics of *Lythrum salicaria* L-Implications for control of this species in North America. Aquatic Botany **38**:303-309.

Wilcox, D.A. 1989. Migration and control of purple loosestrife (*Lythrum salicaria*, L.) along highway corridors. Environmental Management **13**:365-370.

Wilcove, D. S., D. Rothstein, J. Dubow, A. Phillips and E. Losos. 1998. Quantifying threats to imperiled species in the United States. Bioscience **48:** 607-615.

Wilson, L. M., M. Schwarzlaender, B. Blossey and C. B. Randall. 2004. Biology and biological control of purple loosestrife. USDA Forest Service, FHTET-04-12. 78.

Yakimowski, S. B., H. A. Hager, and C. G. Eckert. 2005. Limits and effects of invasion by the nonindigenous wetland plant *Lythrum salicaria* (purple loosestrife): a seed bank analysis. Biological Invasions **7**:687-698.

Yeates A. G., S. S. Schooler, R. J. Garono, Y. M. Buckley. 2012. Biological control as an invasion process: disturbance and propagule pressure affect the invasion success of *Lythrum salicaria* biological control agents. Biological Invasions. **14:** 255–271.

Zamora, R., J. A. Hódar, and J. M. Gómez. 1999. Plant-herbivore interaction: Beyond a binary vision. Pages 677-718 *in* F. Pugnaire and F. Valladares, editors. Functional plant ecology. Marcel Dekker Inc., New York, USA.

Zangerl, A. R., J. G. Hamilton, T. J. Miller, A. R. Crofts, K. Oxborough, M. R. Berenbaum, and E. H. de Lucia. 2002. Impact of folivory on photosynthesis is greater than the sum of its holes. Proceedings of the National Academy of Sciences of the United States of America **99**:1088-1091.

Zehnder, G., A. M. Encill and J. Spreese. 1995. Action thresholds based on plant defoliation for management of colorado potato beetle (Coleoptera: Chrysomelidae) in Potato. Journal of Economic Entomology **88:**155-161.

Table 2.1: Names, codes and coordinates of study sites.

Site		Coordinates	
Name	Code	Latitude	Longitude
Deep River	DPR	46.0790	-77.4790
Algonquin Park #1 (Lookout)	LOO	45.5811	-78.4057
Matawachen	MAT	45.2031	-77.2248
Algonquin Park #2	MOO	45.5427	-78.6657
Highway X	POR	45.3635	-77.6901
Albion Rd.	ALB	45.3156	-75.6209
Deacon	DEA	45.6025	-77.3907
Roger Stevens Dr.	ROG	45.0321	-75.8882
Russel Rd.	RUS	45.3729	-75.4692
Westmeath	WES	45.7980	-76.8890
Whitney	WHI	45.4926	-78.2165
Glen Tay	GLE	44.8833	-76.3000
Jebb's Creek	JEB	44.8571	-76.1792
Kempville	KEM	45.0220	-75.6876
Lombardy	LOM	44.8070	-76.0908
Numogate	NUM	44.9500	-75.9833
Pakenham	PAK	45.3333	-76.2833
Pembroke	PEM	45.8389	-77.1590

Table 2.2: The number of years each site has been exposed to *Galerucella*, how it has been classified categorically and the mean (± SE) site *Galerucella* density and *Nanophyes* density from each visit (June and July).

Site	Years of *Galerucella* exposure [1]	*Galerucella* Colonization History	Herbivore Density (no. / stem)				
			Galerucella (*n* = 15) June	*Galerucella* (*n* = 30) July	*Galerucella* June + July averaged [2]	*Nanophyes* (*n* = 15) June	*Nanophyes* (*n* = 30) July [3]
DPR	0	*Galerucella*	0	0	0	0.07 ± 0.07	1.00 ± 0.48
LOO	0	Free	0	0	0	0	0
MAT	0	(GF)	0	0	0	0	1.40 ± 0.54
MOO	0		0	0	0	0	0.03 ± 0.03
POR	0		0	0	0	0	0
ALB	8	Intermediate	1.47 ± 0.40	6.77 ± 1.30	4.12 ± 2.65	0	2.73 ± 0.82
DEA	14	Colonization	1.53 ± 0.72	0.23 ± 0.09	0.88 ± 0.65	0.47 ± 0.32	0.37 ± 0.13
ROG	13	(IC)	2.2 ± 0.82	1.37 ± 0.71	1.78 ± 0.42	0	0.07 ± 0.07
RUS	8		1.2 ± 0.34	0	0.60 ± 0.60	0.133 ± 0.133	1.40 ± 0.36
WES	13		1.93 ± 0.58	10.47 ± 5.76	6.20 ± 4.27	0	0.57 ± 0.19
WHI	1		0.33 ± 0.27	0.47± 0.32	0.39 ± 0.07	0	0
GLE	21	Early	6.73 ± 3.64	3.7 ± 1.33	5.22 ± 1.52	0.07 ± 0.07	0.57 ± 0.28
JEB	17	Release	0.47 ± 0.22	0.23 ± 0.14	0.35 ± 0.12	0.07 ± 0.07	0.65± 0.24
KEM	16	(ER)	0.53 ± 0.47	0	0.27 ± 0.27	0	0.2 ± 0.15
LOM	21		3.33 ± 0.64	16.66 ± 3.89	10.00 ± 6.67	0	1.07 ± 0.75
NUM	21		1.33 ± 0.29	1.33 ± 0.27	1.33 ± 0.00	0	0
PAK	21		5.53 ± 2.15	7.76 ± 2.14	6.65 ± 1.12	0.07 ± 0.07	1.1 ± 0.43
PEM	18		2.40 ± 0.83	22.37 ± 5.83	12.38 ± 9.98	0	1.3 ± 0.42

[1] Entries in this column for Intermediate Colonization have been estimated, see methods.

[2] To obtain a single measure of *Galerucella* density, data from the June and July visits were averaged. This column was used for all analyses involving site *Galerucella* density.

[3] For *Nanophyes* density, only July measurements were used as this was peak weevil time and the counts were deemed more reliable than those gathered in the June. This column was used for all analyses involving site *Nanophyes* density.

Table 2.3: Site means (±SE) for plant traits, categorized according to colonization history.

	Lythrum salicaria					
Site	Final height (cm)[1]	Above-ground biomass (g)[1]	Above /below-ground biomass[1]	Percentage of plants flowering [2]	No. inflorescences / stem height [2]	Inflorescence length (cm) / stem height [2]
DPR	119.92 ± 6.41	15.84 ± 3.62	0.93 ± 0.14	83.3	0.059 ± 0.001	0.433 ± 0.078
LOO	90.00 ± 6.03	7.67 ± 3.24	0.83 ± 0.10	66.7	0.024 ± 0.006	0.128 ± 0.020
MAT	94.50 ± 8.33	12.92 ± 2.98	0.69 ± 0.08	43.3	0.018 ± 0.006	0.047 ± 0.018
MOO	111.08 ± 8.47	21.69 ± 6.03	1.15 ± 0.10	63.3	0.025 ± 0.003	0.163 ± 0.025
POR	116.33 ± 7.59	26.39 ±14.91	1.07 ± 0.15	40	0.021 ± 0.004	0.217 ± 0.054
Mean ±SE	**106.37 ± 3.57**	**16.90 ± 3.40**	**0.94 ± 0.06**	**59.33 ± 7.99**	**0.029 ± 0.003**	**0.198 ± 0.023**
ALB	119.00 ± 6.67	12.88 ± 3.03	0.54 ± 0.11	73.3	0.036 ± 0.007	0.207 ± 0.035
DEA	102.50 ± 6.21	19.12 ± 3.23	1.52 ± 0.26	60	0.039 ± 0.006	0.224 ± 0.032
ROG	101.58 ± 5.76	23.58 ± 6.95	1.19 ± 0.17	90	0.056 ± 0.007	0.419 ± 0.072
RUS	155.42 ± 6.13	72.36± 16.82	1.14 ± 0.37	93.3	0.057 ± 0.009	0.414 ± 0.074
WES	154.25 ± 5.81	42.75 ± 6.50	0.53 ± 0.07	40	0.061 ± 0.010	0.229 ± 0.031
WHI	101.92 ± 6.66	12.54 ± 4.58	1.14 ± 0.10	76.7	0.017 ± 0.002	0.174 ± 0.023
Mean ±SE	**122.44 ± 3.72**	**30.53 ± 4.13**	**1.01 ± 0.09**	**72.22 ± 8.10**	**0.044 ± 0.003**	**0.278 ± 0.021**
GLE	103.92±3.98	24.07 ± 4.54	0.83 ± 0.10	6.67	0.040 ± 0.007	0.117 ± 0.022
JEB	128.75±9.52	22.22 ± 3.47	0.83 ± 0.07	80	0.033 ± 0.006	0.220 ± 0.037
KEM	98.92±4.13	3.74 ± 0.44	0.80 ± 0.18	86.7	0.019 ± 0.003	0.140 ± 0.014
LOM	133.42±3.56	26.97 ± 3.75	0.83 ± 0.10	26.7	0.067 ± 0.026	0.247 ± 0.057
NUM	59.00±2.88	1.22 ±0.14	0.31 ± 0.07	13.3	0.016 ± 0.005	0.044 ± 0.011
PAK	120.75±6.68	61.87 ± 11.29	1.31 ± 0.26	20	0.044 ± 0.014	0.224 ± 0.074
PEM	134.50±6.62	35.20 ± 7.28	1.01 ± 0.30	23.3	0.046 ± 0.012	0.186 ± 0.038
Mean ±SE	**111.32±3.46**	**25.04 ± 2.93**	**0.90 ± 0.08**	**36.67 ± 12.32**	**0.038 ± 0.005**	**0.168 ± 0.0162**
Overall Mean ±SE	**113.65 ± 2.12**	**24.61 ± 2.05**	**0.95 ± 0.05**	**54.81 ± 6.72**	**0.038 ± 0.002**	**0.213 ± 0.012**

[1] Response variable used to assess productivity, collected in September (n=12)
[2] Response variable used to assess reproductive output, collected in July (n=30)
[3] Response variable used to assess reproductive output, collected in September (n=12)

Table 2.3 (continued):

Site	*Lythrum salicaria*		
	No. Fruit / plant [3]	No. fruit /g above-ground biomass [3]	Fruit density[3]
DPR	600.33 ± 128.77	40.18 ± 4.10	4.46 ± 0.51
LOO	318.67 ± 174.19	33.52 ± 3.11	4.42 ± 0.44
MAT	99.25 ± 31.72	18.24 ± 8.03	2.60 ± 0.48
MOO	580.83 ± 168.41	24.86 ± 2.90	5.52 ± 0.94
POR	524.67 ± 264.60	23.08 ±1.24	5.00 ± 0.28
Mean ±SE	**424.75 ± 77.93**	**27.98 ± 2.19**	**4.40 ± 0.28**
ALB	86.50 ± 28.39	5.97 ± 1.26	1.16 ± 0.22
DEA	528.83 ± 141.23	29.31± 4.50	5.56 ± 0.47
ROG	1160.00 ± 88.63	41.17 ± 4.74	4.38 ± 0.40
RUS	1295.50 ±391.04	16.16 ± 1.63	2.51 ± 0.24
WES	611.50 ± 103.52	14.97± 1.69	3.11 ± 0.48
WHI	509.55 ± 200.14	38.62 ± 5.06	6.75 ± 0.41
Mean ±SE	**701.31 ± 120.71**	**24.37 ± 2.07**	**3.9 ± 0.27**
GLE	311.50 ± 98.32	10.74 ± 2.64	3.03 ± 0.39
JEB	672.33 ± 141.54	29.65 ± 4.11	4.53 ± 0.50
KEM	90.17 ± 20.72	24.12 ± 3.59	3.44 ± 0.36
LOM	307.33 ± 81.39	9.98 ± 0.44	1.46 ± 0.24
NUM	20.67 ± 6.65	18.00 ± 5.48	2.00 ± 0.54
PAK	622.18 ± 250.03	9.11 ± 2.11	1.36 ± 0.23
PEM	422.83 ± 199.69	7.51 ± 2.51	0.98 ± 0.31
Mean ±SE	**346.29 ± 55.91**	**15.49 ± 1.51**	**2.40 ± 0.19**
Overall Mean ±SE	**486.07 ± 51.38**	**21.72 ± 1.11**	**3.46 ± 0.15**

[1] Response variable used to assess productivity, collected in September (n=12)
[2.] Response variable used to assess reproductive output, collected in July (n=30)
[3.] Response variable used to assess reproductive output, collected in September (n=12)

Table 2.4: Pearson correlation matrix of six *Lythrum* plant traits.

Trait	Plant height	No. inflorescences	Total inflorescence length	No. fruit	Above-ground biomass	Below-ground biomass
Plant height	1					
No. inflorescences	0.7517**	1				
Total inflorescence length	0.7818**	0.6281**	1			
No. fruit	0.5401*	0.9616**	0.7546**	1		
Above-ground biomass	0.7396**	0.9023**	0.9384**	0.6394**	1	
Below-ground biomass	0.7924**	0.6655**	0.7456**	0.4666	0.7903**	1

*Correlation is significant at the $P < 0.05$ level.
**Correlation is significant at the $P < 0.01$ level.

Table 2.5: *Lythrum* stem density, number of plant families and species identified at each site along with mean (± SE) quadrat species richness observed at the June and July site visits.

Site	*Lythrum* density (no. stems/ quadrat)	Total no. plant families identified over season	Total no. plant species identified over season	Species Richness	
				Quadrats (June) $n = 15$	Quadrats (July) [1] $n = 15$
DPR	5.07 ± 1.14	17	50	6.33 ± 0.45	8.80 ± 0.51
LOO	6.40 ± 1.77	15	53	6.60 ± 0.58	8.33 ± 0.57
MAT	6.27 ± 1.99	17	40	5.60 ± 0.32	5.27 ± 0.31
MOO	4.73 ± 0.93	15	50	6.93 ± 0.60	6.67 ± 0.62
POR	5.60 ± 1.52	11	27	3.53 ± 0.22	4.87 ± 0.36
ALB	12.40 ± 2.54	10	30	6.53 ± 0.32	6.87 ± 0.36
DEA	4.26 ± 0.88	9	30	5.53 ± 0.51	5.73 ± 0.64
ROG	9.00 ± 2.65	11	40	5.53 ± 0.61	5.87 ± 0.62
RUS	9.40 ± 3.90	14	45	7.67 ± 0.41	5.00 ± 0.39
WES	1.33 ± 0.50	15	44	4.40 ± 0.50	5.20 ± 0.46
WHI	15.93 ± 2.15	15	39	4.53 ± 0.52	4.93 ± 0.67
GLE	1.47 ± 0.51	10	25	5.87 ± 0.26	5.87 ± 0.26
JEB	3.47 ± 1.06	8	18	3.27 ± 0.25	7.20 ± 1.12
KEM	9.87 ± 2.03	12	35	5.93 ± 0.36	6.13 ± 0.47
LOM	21.07 ± 3.04	13	33	4.87 ± 0.35	4.07 ± 0.40
NUM	6.54 ± 1.78	15	41	5.53 ± 0.77	6.07 ± 0.77
PAK	9.07 ± 1.76	18	48	5.00 ± 0.45	4.93 ± 0.52
PEM	7.87 ± 2.46	15	39	5.73 ± 0.72	6.20 ± 0.84

[1] Indicates column of data used for all analyses involving average site quadrat richness species richness.

Table 2.6: Model selection for A) number of inflorescences per plant and B) total length of inflorescences, based on AICc. All models included Stem height, *Galerucella* per stem, *Nanophyes* per stem, and Site as a random effect. Non-selected models also contain interactions: *Gal:Height* (A1, B1) and *Gal:Nan* (A1, A2, B1, B2). Bolded models (A3, B3) are identified as being the 'best' models.

Model	Number of parameters	logLik	AICc	ΔAICc
A1	8	164.47	180.73	*17.12*
A2	7	164.77	178.97	15.36
A3	**6**	**151.45**	**163.61**	**0.00**
B1	8	559.76	693.42	133.61
B2	7	559.95	574.15	14.34
B3	**6**	**547.65**	**559.81**	**0.00**

Table 2.7: Parameter estimates for the best models (Models A3, B3 from Table 2.6) predicting the number of inflorescences per stem and the total length of inflorescences (cm) per stem. This model included the fixed effects of stem height, as well as the number of *Galerucella* per stem and the number of *Nanophyes* per stem. Site was also included as a random factor within the model as it accounted for the variation within site. No interaction terms that were explored improved the model. Estimates are given on a log-scale relative to the intercept.

Model	Dependent variable	Variable	Estimate	SE
A3	$\log_{10}$	Intercept	-0.0201	0.0508
	(No. Inflorescences +1)	Stem height	0.0072	0.0005
		Galerucella per stem	0.0050	0.0009
		Nanophyes per stem	0.0337	0.0063
		Site (among sites)	0.0130	0.0053
		Residual (within site)	0.0681	0.0042
B3	$\log_{10}$	Intercept	-0.2878	0.0853
	(Inflorescence length +1)	Stem height	0.0137	0.0007
		Galerucella per stem	0.0042	0.0013
		Nanophyes per stem	0.0375	0.0091
		Site (among sites)	0.0601	0.0225
		Residual (within site)	0.0397	0.0087

Figure 1.1: Known *Galerucella* (*G. calmeriensis* and *G. pusilla*) releases (pre-1996) in Ontario. (McKenzie, pers. comm.). Two releases in Timmins, ON not shown.

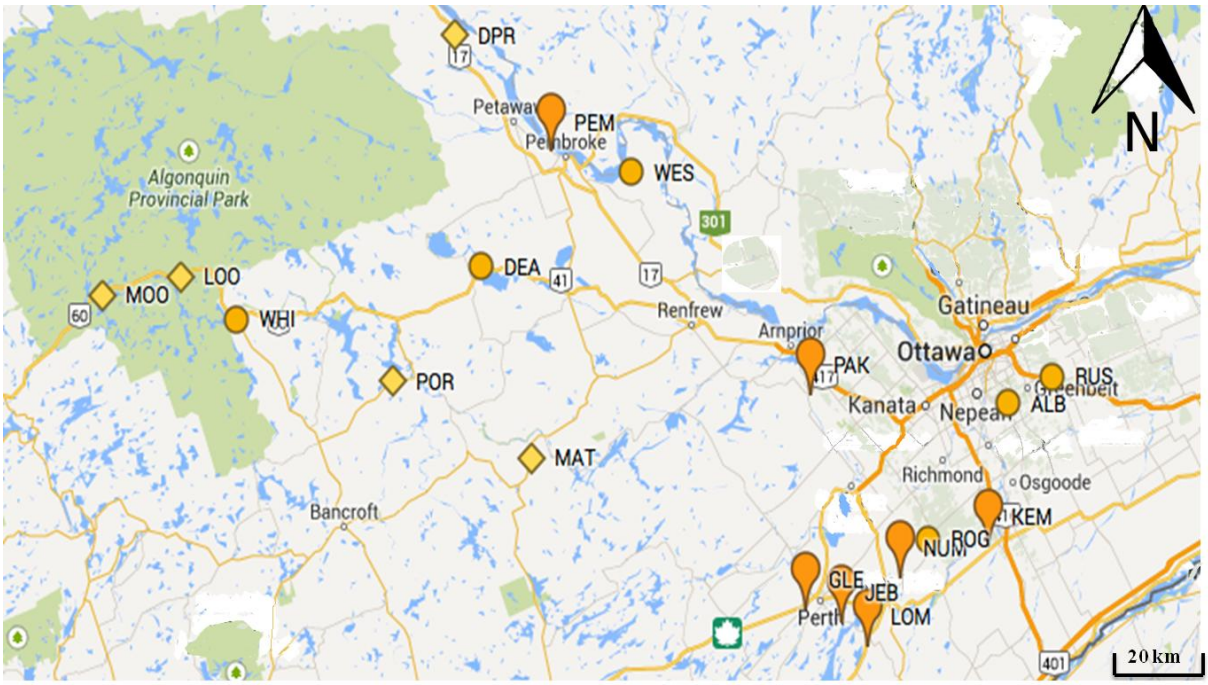

Figure 2.1: Map of study sites. *Galerucella*-free sites are indicated by diamonds, intermediate colonization sites are indicated with circles and early release sites are indicated with pins. Exact coordinates can be found in Table 2.1.

a)

d)

b)

e)

c)

f)

Figure 2.2: Photos of *Galerucella calmeriensis* a) mating adults, b) several egg masses and c) larvae and feeding damage to *Lythrum*. Photos of *Nanophyes marmoratus* adults d) female, e) male and f) damaged terminal *Lythrum* inflorescence.

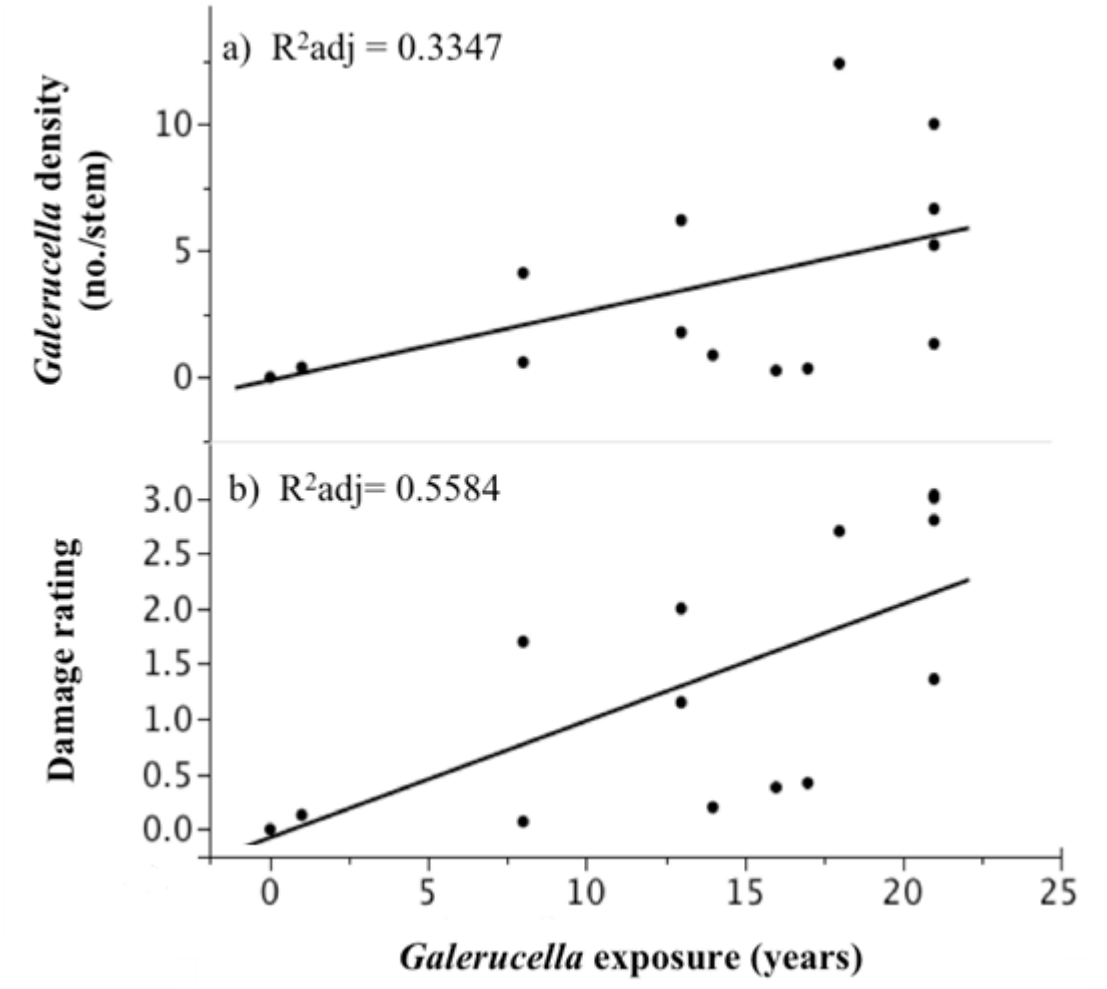

Figure 2.3: Linear regressions showing the associations between the number of years of *Galerucella* exposure and the mean *Galerucella* density ($F = 9.5548$, $df = 17$, $P < 0.007$) and damage rating per site ($F = 22.50$, $df = 17$, $P < 0.0002$).

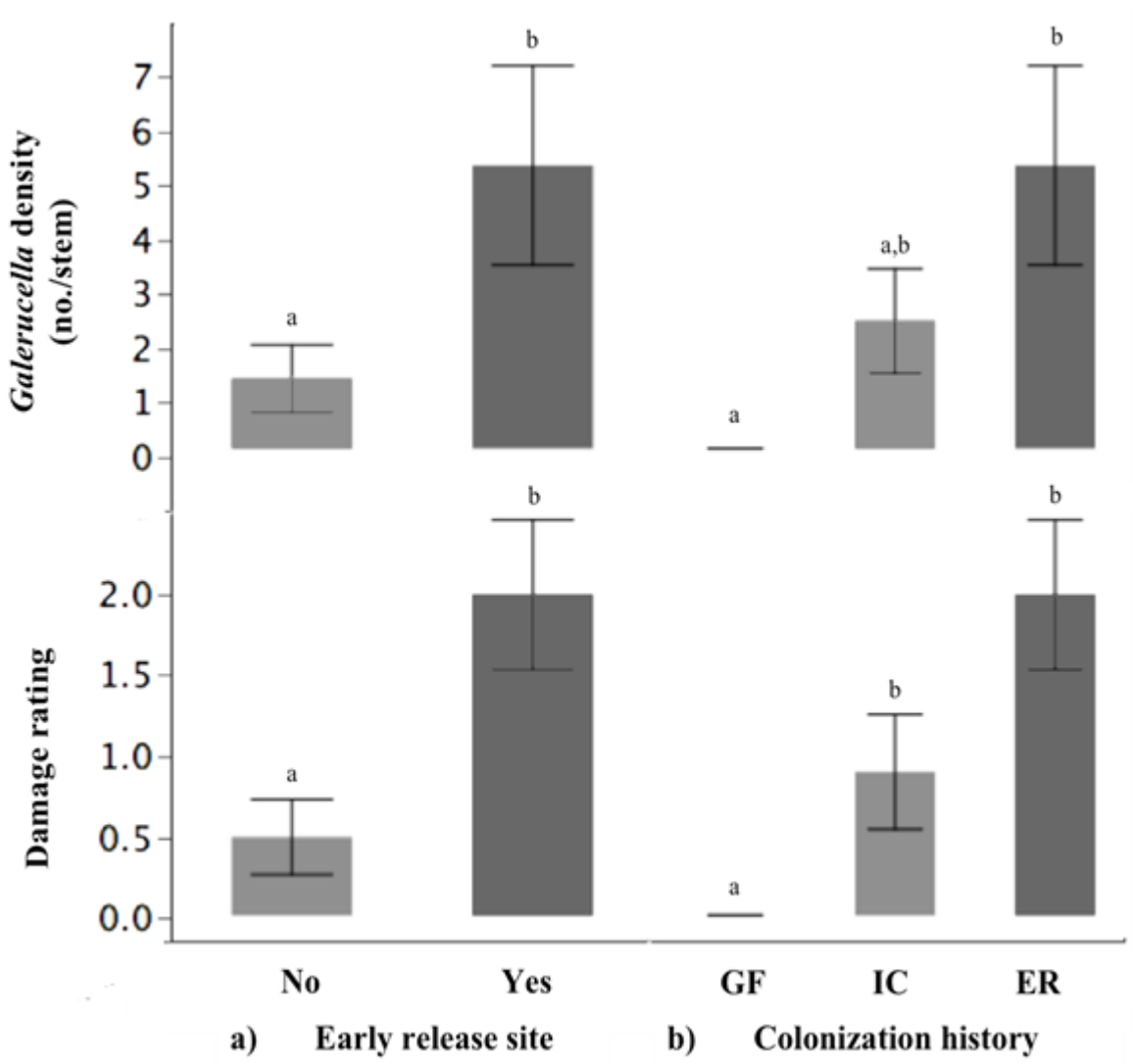

Figure 2.4: a) Mean (±SE) *Galerucella* density and damage ratings for sites categorized as early *Galerucella* release sites (or not) b) Mean (±SE) *Galerucella* density and damage ratings for sites categorized according to colonization history (*Galerucella*-free sites (GF), intermediate colonization (IC) and early (*Galerucella*) release (ER) sites). Note: Bars that are not connected by the same letter are significantly different, based on all pairs comparison using Tukey-Kramer HSD ($\alpha = 0.05$).

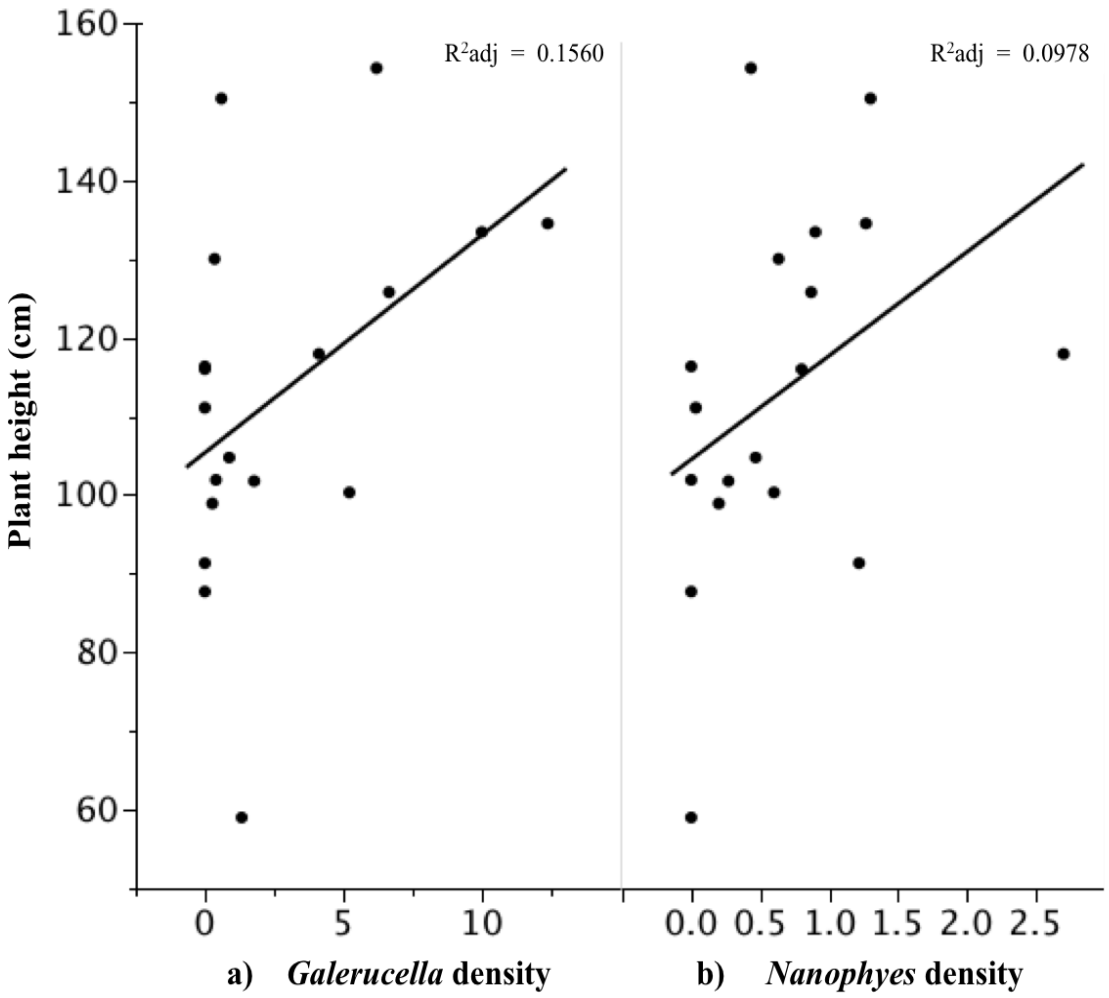

Figure 2.5: Linear regressions showing the associations between mean plant height and the a) *Galerucella* density (F = 4.1413, df = 17, P = 0.0588) and b) *Nanophyes* density (F = 2.8433, df = 17, P = 0.1112) at each site.

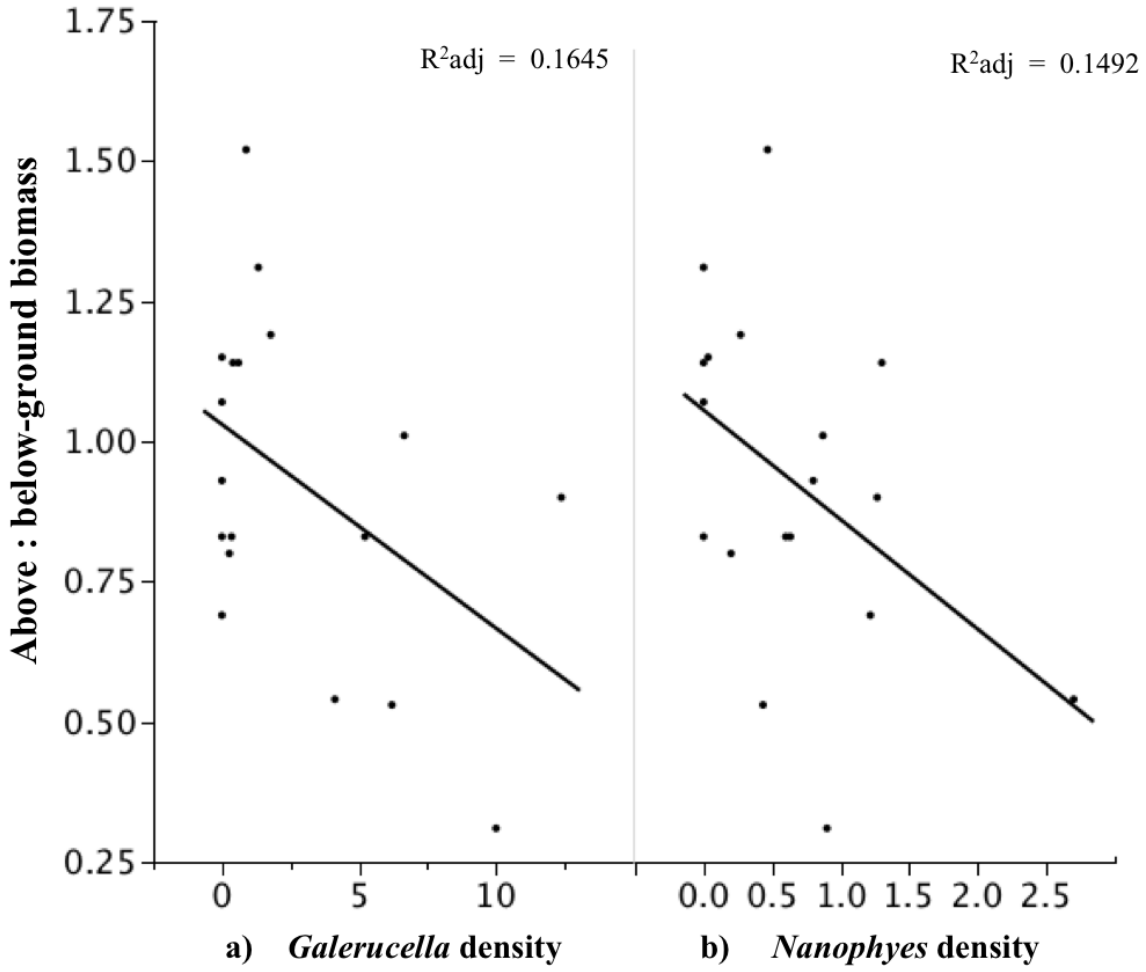

Figure 2.6: Linear regression showing the association between mean the ratio of above to below-ground biomass and a) *Galerucella* density ($F = 4.3487$, $df = 17$, $P = 0.0534$) and b) *Nanophyes* density ($F = 3.9804$, $df = 17$, $P = 0.0634$) for each site.

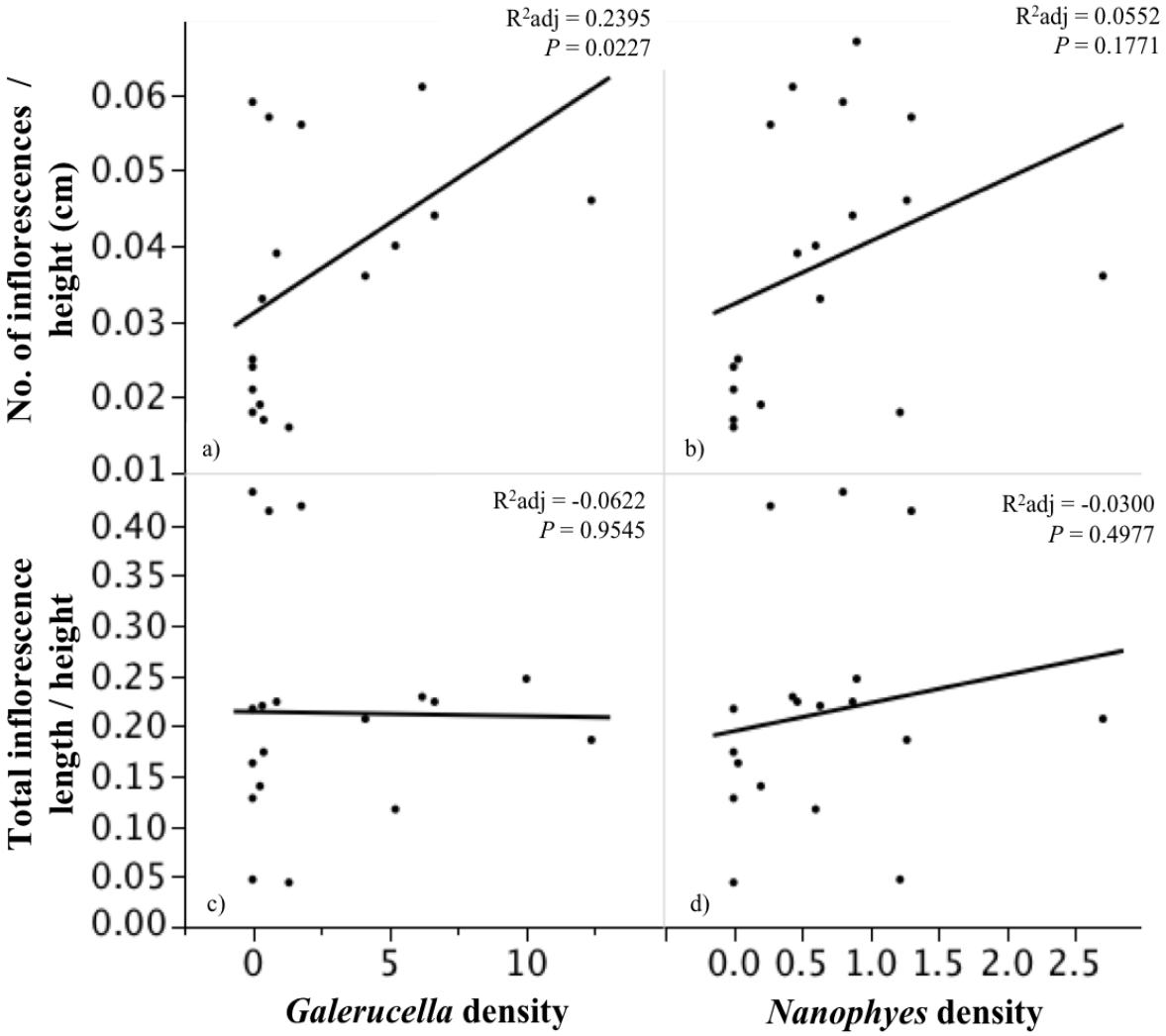

Figure 2.7: Linear regressions showing the association between site means (July, *n* = 30) of a) number of inflorescences (per plant height) and *Galerucella* density, b) number of inflorescences (per plant height) and *Nanophyes* density, c) the total inflorescence length (per plant height) and *Galerucella* density and d) the total inflorescence length (per plant height) and *Nanophyes* density.

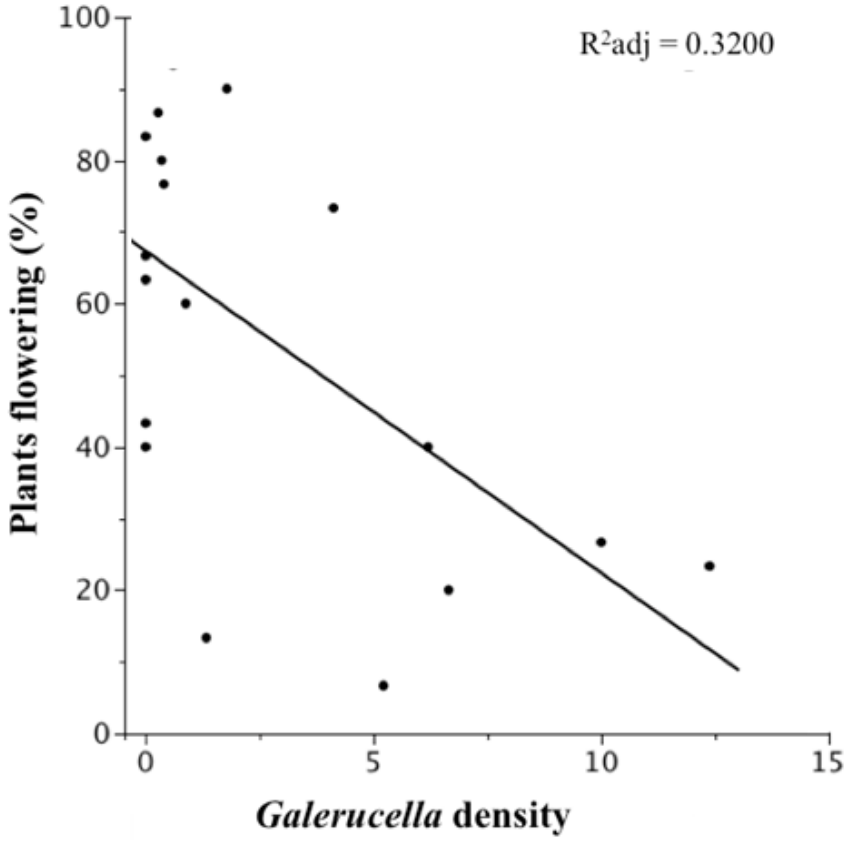

Figure 2.8: Linear regression showing an association between the percentage of plants flowering at a site (late July) and the *Galerucella* density (F = 8.9828, df = 17, P = 0.0085).

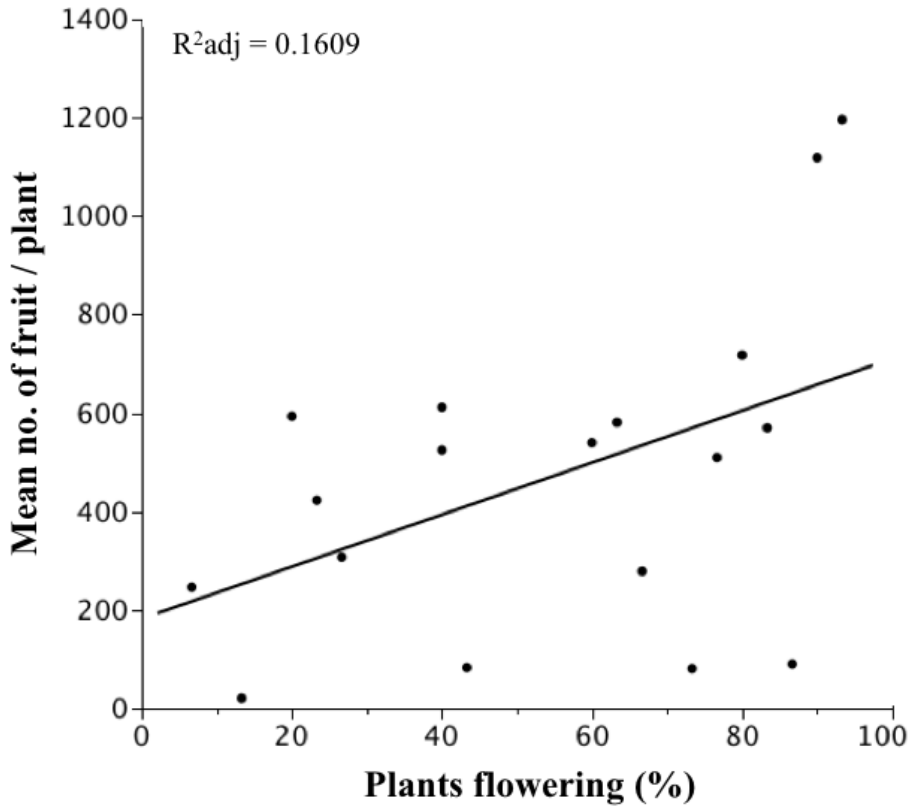

Figure 2.9: Linear regressions showing the association between the mean number of fruit produced per plant per site and the floral phenology (percentage of plants flowering at the July visit) at that site ($F = 4.2594$, $df = 17$, $P = 0.0556$).

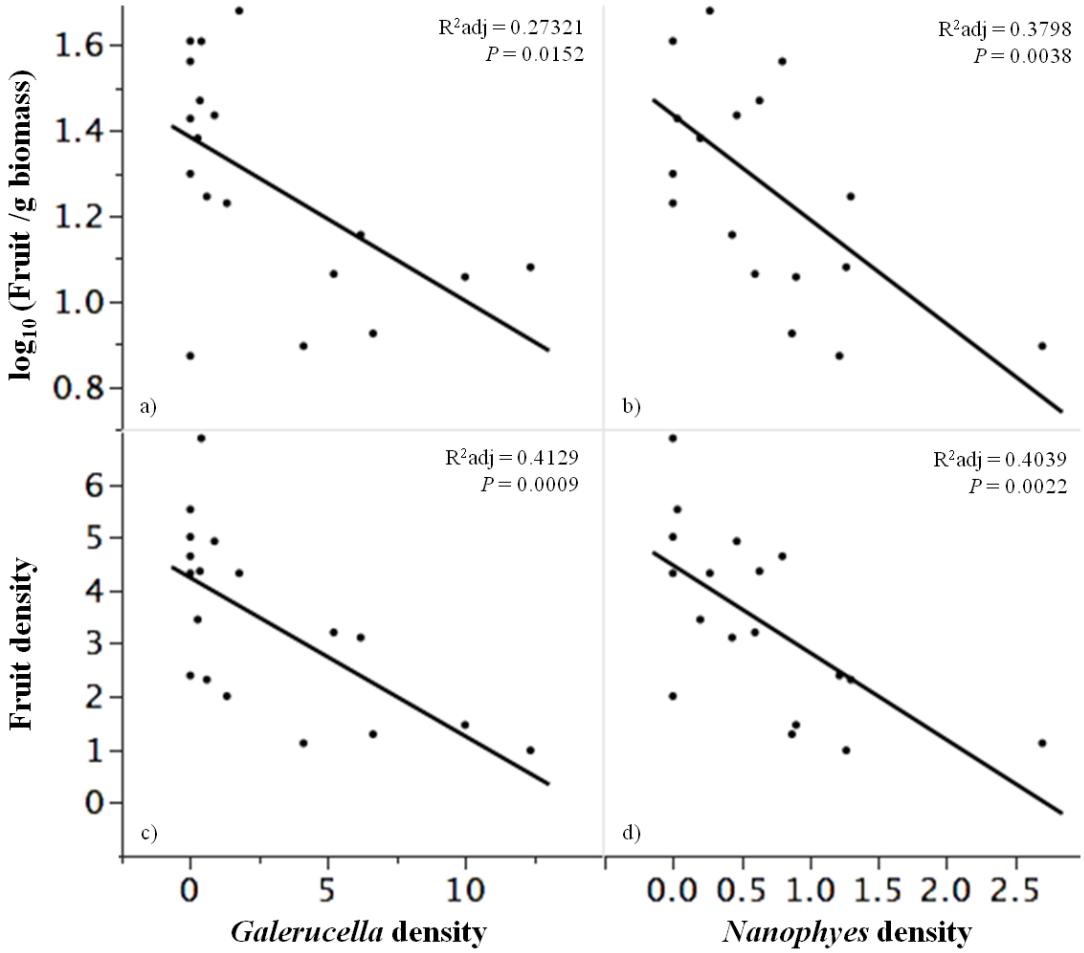

Figure 2.10: Linear regressions showing the associations between site means (*df* =17) of a) the $\log_{10}$ (number of fruit per gram of above-ground biomass) and *Galerucella* density, b) the $\log_{10}$ number of fruit per gram of above-ground biomass and *Nanophyes* density, c) fruit density (number of fruit per cm infructescence) and *Galerucella* density and d) fruit density (number of fruit per cm infructescence) and *Nanophyes* density.

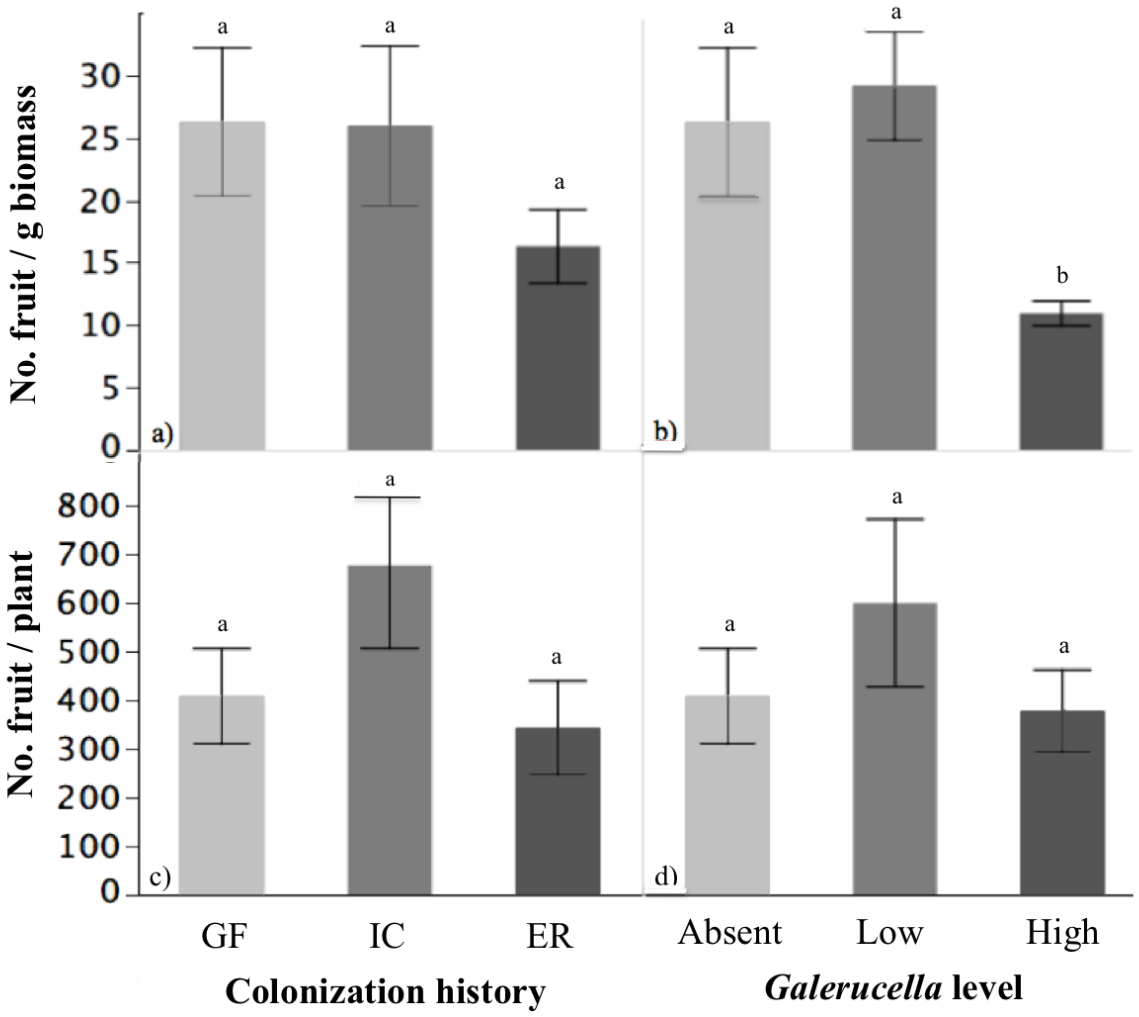

Figure 2.11: Mean (±SE) number of fruit per gram of above-ground biomass for three categories of a) *Galerucella* colonization history and b) present-day *Galerucella* levels and mean (±SE) number of fruit per plant according to c) *Galerucella* colonization history and d) present-day *Galerucella* levels. Note: Bars that are not connected by the same letter are significantly different ($P < 0.05$). *Galerucella* level catergories are: absent (0 *Galerucella* per stem), low (> 0 < 2 *Galerucella* per stem), and high (> 4 *Galercucella* per stem).

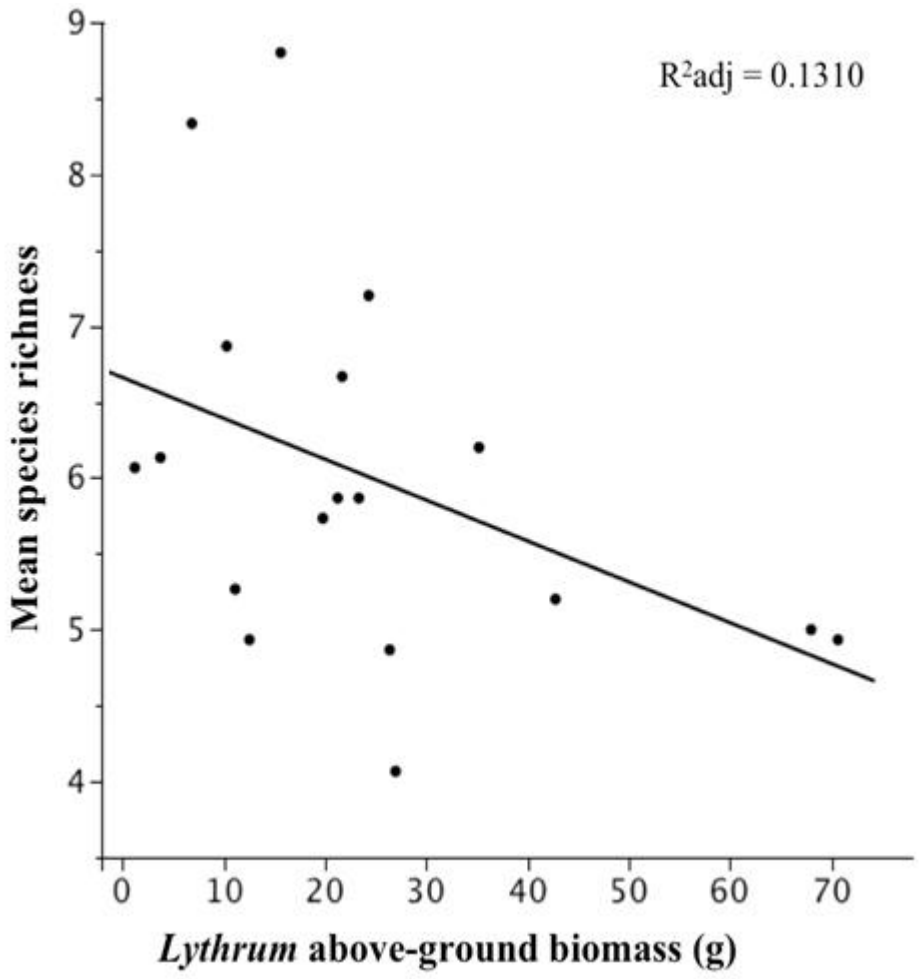

Figure 2.12: Linear regression showing the association between mean (n = 15) quadrat plant species richness and mean above-ground biomass (g) for *Lythrum* for each site (F = 3.56, df = 17, P = 0.0778).

CHAPTER 3: Conclusions and Future Directions

The work presented here demonstrates that feeding on Ontario populations of *Lythrum salicaria* by two biological control agents, *Galerucella calmeriensis and Nanophyes marmoratus,* has the capacity to decrease the reproductive output of this invasive perennial. I also detected an association between *Galerucella* density and delayed floral phenology, a feature important to reproductive output and therefore plant population dynamics.

Although increasing *Galerucella* density (or damage) is associated with an increase in the total number of inflorescences per plant, this does not necessarily translate into increased fruit set (Schat and Blossey (2005). This indicates that traits important for reproductive output (e.g., inflorescence length and hence the number of flowers) can vary with the type and intensity of herbivore damage. One might also expect to find indirect effects such as altered interactions with pollinators or effects on plant community composition and richness as a result of herbivory. Indeed, we know that plant responses to herbivory vary depending on the intensity and location of feeding damage along with environmental factors (Lande and Arnold 1983, Doak 1992) and/or genetic differences among populations (e.g., Halpern et al. 2010, Brody and Irwin 2012).

The lack of a significant effect of herbivory on vegetative productivity is an important piece of the puzzle, as it contrasts reports of reductions in height by Schat and Blossey (2005) and (above-ground) biomass (Bastolová and Kvet 2002). A negative association was detected between allocation to leaves as a proportion of plant biomass and *Galerucella* density (similar to Schat and Blossey (2005)), but it was not statistically significant.

I am unfortunately unable to disentangle the effects of the two herbivores found in varying densities at my 18 sites. Experimentally examining the effects of the individual herbivores would make an excellent next step. Future studies could also focus on identifying

the specific plant traits most attractive to each herbivore. A further understanding of the effects of herbivory on *Lythrum* reproductive success may be gained by examining fitness of the next generation of plants.

As the Canadian distribution of *Nanophyes* has only been partially described (Douglas 2013, St. Louis 2013), a more thorough search for the extent of its range and path(s) of introduction would be worthwhile. It is possible that since *Nanophyes* is such a strong disperser and establisher it has travelled to and resides in more northern *Lythrum* populations, where its success and impacts are currently unknown.

As in Hambäck et al. (2000), who found that the presence of the plant *Myrica gale*, reduced *Galerucella* damage to *Lythrum*, likely by somehow concealing its presence, examining the influence of the plant community or the presence of a particular plant within the community exposed to a biological control program could also prove informative. For example, neighbouring plants could compete with a target pest plant species, possibly altering their responses to herbivory (e.g., Hjälten et al. 1973, Boege 2010). Some authors have found that the introduction of a biological control agent is associated with an increase in species richness or a return of balance in the invaded ecosystem (Huffaker and Kennett 1959, Bellows 2001, Albright 2004). Follow up studies could reveal detectable changes in plant community.

The work presented in this thesis demonstrates that feeding by two biological control agents can affect rates of fruit production in *Lythrum* and adds an important long-term assessment of the biological control of this invasive species.

References

Albright M. F, W. N. Harman, S. S. Fickbohm, H. Meehan, S. Groff, and T. Austin. 2004. Recovery of native flora and behavioural responses of *Galerucella sp*. following biocontrol of purple loosestrife. The American Midland Natualist. **152**:248-254.

Bastolová D. and J. Kvet 2002. Differences in dry weight partitioning and flowering phenology between native and non-native plants of purple loosestrife (*Lythrum salicaria* L.). Flora 197:332-340.

Bellows, T. S. 2001. Restoring population balance through natural enemy introductions. Biological Control **21**:199–205.

Brody, A. K. and R. E. Irwin. 2012. When resources don't rescue: Flowering phenology and species interactions affect compensation to herbivory in *Ipomopsis aggregata*. Oikos **121**:1424-1434.

Boege, K. 2010. Induced responses to competition and herbivory: Natural selection on multitrait phenotypic plasticity. Ecology **91**:2628-2637.

Doak, D. F. 1992. Lifetime impacts of herbivory for a perennial plant. Ecology **73**:2086-2099.

Douglas, H., P. Bouchard, R.S. Anderson, P. de Tonnancour, R. Vigneault, R. and R.P. Webster. 2013. New Curculionoidea (Coleoptera) records for Canada. Zookeys **309**:13-48.

Halpern, S. L., L. S. Adler, and M. Wink. 2010. Leaf herbivory and drought stress affect floral attractive and defensive traits in *Nicotiana quadrivalvis*. Oecologia **163**:961-971.

Hambäck, P. A., J. Ågren, and L. Ericson. 2000. Associational resistance: Insect damage to purple loosestrife reduced in thickets of sweet gale. Ecology **81**:1784-1794.

Hjälten, J., K. Danell, and L. Ericson. 1993. Effects of simulated herbivory and intraspecific competition on the compensatory ability of birches. Ecology **74**:1136-1142.

Huffaker, C. B. and C. E. Kennett. 1959. A ten-year study of the vegetational changes associated with biological control of Klamath weed. Journal of Range Management **12**:69-82.

Lande R. and S. J. Arnold.1983. The measurement of selection on correlated characters. Evolution **76**:1210-1226.

Schat, M. and B. Blossey. 2005. Influence of natural and simulated leaf beetle herbivory on biomass allocation and plant architecture of purple loosestrife (*Lythrum salicaria* L.). Environmental Entomology **34**:906-914.

St. Louis, E. 2013. New Ontario records for *Nanophyes m. marmoratus* (Coleoptera:

Brentidae) introduced into North America for the classical biological control of purple loosestrife. Journal of the Entomological Society of Ontario. **144**:19-123.