Variation in Parasitism in *Galerucella calmariensis* (Coleoptera:Chrysomelidae), an Introduced Biocontrol Agent: Enemy Release and Sex Bias in Parasitism

by Kathryn J. Norman

A thesis submitted to

the Faculty of Graduate Studies and Research

in partial fulfillment of the requirements for the degree of

Master of Science.

Department of Biology

Carleton University

Ottawa, Ontario

Your file Votre référence
ISBN: 978-0-494-18371-7
Our file Notre référence
ISBN: 978-0-494-18371-7

ABSTRACT

The enemy release hypothesis (ERH) is one of the most-studied explanations for the success of invasive species, yet the results of these studies are variable and often contradictory. Failure to properly consider confounding variables may be the cause of this. Variables such as host sex, age, and population density, as well as relative date may all impact parasitism rates but are often not considered in studies testing the ERH. Furthermore, invasive species may accumulate parasites faster than expected, which could further confound results.

This study attempted to clarify the effects of these factors using the introduced biocontrol beetle *Galerucella calmariensis*. This species was surveyed across a broad portion of it's introduced range for parasites and fungal pathogens. Three varieties of parasite and six fungal species were found. Significant effects of host sex, age, and relative date were found, along with evidence of rapid parasite accumulation, indicating that these factors should be considered in studies of the ERH.

Acknowledgements

I would like to thank my two supervisors for their assistance with this project. I was accepted into graduate school as a member of the Forbes' lab, inspired by Mark's enthusiasm for research. Later Naomi Cappuccino became my co-supervisor when my project took a turn towards her area of expertise. Naomi's calm approach provided an excellent counterpart to Mark's excitement, and the couch in her lab provided an excellent place to sit and think quietly.

I would like to thank the many people who provided feedback and insights during the course of what turned out to be a long and circuitous data analysis process. Pam Rutherford, Tonia Robb, and Felix Eigenbrod provided invaluable help when the program R refused to do my bidding, and Paul Smith and Tom Sherrat contributed helpful statistical advice.

My office-mate Yemisi Dare was an excellent sounding board for ideas and a co-conspirator in all my grad-school survival schemes.

All the members of the Forbes' lab during my time here have been helpful, graciously coming to lab meetings and hearing my proposals. Thanks to Pam Rutherford, Selma Mautner, Arash Rashed, Tonia Robb, Yemisi Dare, Paul Smith, Stacey Robinson, Kathy Cook, Lynne Rasmusen, Jennilee Gobin, and Joanna James. Thanks as well to unofficial lab members Stacey Lee- Jenkins and Chris Beatty.

My family, though sometimes maddening, always supported me during my studies and instilled me with the love of science from an early age.

And finally, I wouldn't have made it through the final stages of writing without the calming voice of reason and encouragement of my fiancé, Eric, and a certain fluffy feline called Pippin. Thanks guys.

Table of Contents

List of Tables

List of Figures

Introduction

Numerous species of plants and animals are constantly being introduced into novel habitats and ranges around the world. The majority of these species fail to survive or fail to thrive, but a small percentage of these, termed 'invasive species' flourish in their introduced ranges. These species often become serious ecological or economic problems, and a great deal of time and effort has been put into discovering what differentiates the species that thrive from those that fail. This field of research has been termed 'invasion biology'.

From its inception, the term 'invasive' has been vague, and today there are many variations of the precise meaning of the term (Richardson et al, 2000). However, invasive species are generally considered to be those that spread from their introduction site and become dominant members of the ecosystem (Davis & Thompson, 2001, Kolar & Lodge, 2001). Most exotic species, upon introduction to a new environment, do not become invasive. The ones that do can have profound impacts on both local ecology and economics, and much research has been devoted to understanding why invasive species become invasive.

The enemy release hypothesis (ERH) has received much attention as a potential explanation for the success of invasive species. It is intuitively appealing, and has been widely tested in the context of plant invaders. It is often considered to be the primary explanation for the success of invasive plants (Mitchell & Power, 2003). This hypothesis now stands as the most widely accepted and most tested explanation. The ERH was developed specifically for invasive plants (Keane & Crawley, 2002, Maron & Vilà, 2001). It states that introduction into a new geographical region is associated with a

decrease in natural enemies, allowing the plant to rapidly increase in abundance and distribution. For plants, natural enemies consist mainly of generalist and specialist herbivores that feed on the plant, but may also include pathogens such as fungi, viruses and bacteria. When exotic animals are considered, natural enemies include predators, pathogens, and parasites/parasitoids.

Often tested in conjunction with the ERH is the biotic resistance hypothesis (BRH) which takes the opposite tack and seeks to explain why most species do *not* become invasive when introduced into a new region. It states that introduction into a new area results in interference by native species, which hinders the ability of the introduced species to become established (Maron & Vilà, 2001). Interference may take the form of native species which occupy the same niche as the exotic species but are better adapted or novel enemies for which the species has no defenses. This of course assumes that abiotic resistances (such as temperature, water and food requirements which may not be met) have already been overcome.

There has been much work done testing the ERH. Much attention has been devoted to determining whether invasive or introduced species will have fewer natural enemies, or suffer less from them, in their introduced range than their native range. Results have been mixed in this regard. Biogeographical studies (comparing one species in its native and introduced range) generally support the ERH, whereas community studies (comparing native and introduced species in the same range) do not (Colautti et al., 2004).

Torchin et al. (2001) examined invasive and native populations of the marine crab *Carcinus maenas* around the world and found that invasive populations had lower

parasitism rates and were larger in size than native populations. Mitchell & Power (2003) compared fungal and viral pathogens on invasive plants in their native and introduced ranges (using the definition of naturalized plants that had become pests). They found an 84% reduction in the number of pathogenic fungal species and a 24% reduction in the number of viral species on average in introduced ranges, and furthermore found a positive correlation between the degree to which a species was reported as a pest and the degree of pathogen release.

Other studies have revealed mixed results. For example, Joshi & Vrieling (2005) found higher growth and reproduction rates and lower defense against specialist herbivores in introduced weed populations, but also higher defense against generalist predators, suggesting that pressure from generalist predators is greater in the introduced range. Likewise, Vilà et al. (2005) found that *Hypericum perforatum* experienced less herbivory and formed denser populations in its introduced range, but that individual plants grew smaller. Torchin et al. (2003) compared 26 introduced animal species in native and introduced ranges and found that parasite diversity and load were lower in the introduced range, but the most common parasites (and therefore probably those that have the most impact on their hosts) still achieved the same prevalence rates.

Another approach has been to test the assumption that in the introduced range, invasive species outperform natives because they experience less of an effect from natural enemies than the native species do. The results have been similarly inconclusive. Dietz et al., (2004) compared the amount of feeding damage on the leaves of native and invasive woody plants and found that natives suffered more damage and leaf loss than non-natives, supporting the ERH. Blaney & Kotanen, (2001) compared the effects of

fungal pathogens on native and invasive seeds in two habitats, and found no difference in seed viability that could be attributed to the fungi, giving results that do not support the ERH. Agrawal & Kotanen (2003), comparing 15 phylogenetically related pairs of native and exotic plants grown in a common garden, found that non-native plants had greater or equal levels of herbivore damage than native plants, contradicting the ERH.

The majority of studies are not experimental, despite stipulations that enemy exclusion experiments are essential for testing the ERH (Keane & Crawley, 2002, Maron & Vilà, 2001). In the only two studies to exclude enemies, support for the ERH is mixed. Blaney & Kotanen (2001) used fungicide to exclude fungal enemies and found no effect, and in another decisive study fungicide and insecticide were used to exclude enemies and demonstrate the enemy release was responsible for the success of the invasive shrub *Clidemia hirta* (DeWalt et al., 2002).

Why such variation? One explanation is that confusion about definitions obscures effects, such as when non-native but relatively benign plants are considered alongside or instead of highly invasive ones. This was the case in Agrawal & Kotanen's study (2003). In one study comparing native and non-native plants, considering the degree of invasiveness of plants led to findings that supported the ERH (Carpenter & Cappuccino, 2005). The importance of considering the degree of invasiveness of non-native species is underscored by the work of Mitchell & Power (2003), where more invasive species were found to have greater enemy release.

Another explanation for the apparently conflicting results of ERH studies is that enemy release does not happen all the time. Invasiveness may have many causes. Some species may experience sufficient enemy release to allow them to become invasive,

whereas others may not experience release to the same degree or may not have other predisposing factors which allow them to exploit this release.

In their review, Colautti et al (2004) found that approximately 60% of studies support the ERH. They also suggest factors that are not often considered which may affect the outcome of such studies. One factor is comparing invasive species in the introduced range to the native population where founding individuals originated, rather than over the entire range. Conversely, exotic species should be considered at more than one place and time in their novel ranges. Another factor that Colautti et al (2004) suggest which may affect the outcome of ERH research is considering the difference between regulatory release and compensatory release. Regulatory release consists of release from enemies that the host is poorly defended against, resulting in an immediate increase in population viability. Compensatory release is different, in that release from enemies that the host is well defended against occurs gradually as resources are reallocated to defense or the host adapts to the novel environment.

One potential source of variation that has not received much attention in the literature is confounding factors. Host related factors such as sex, age and population density, and other factors such as time of season and time since introduction of a non-native host species can all impact parasitism.

Whether host sex influences parasitism and immunity in general has been broadly studied in vertebrates (Poulin, 1996, Zuk & McKean, 1996, Moore & Wilson, 2002). Sex biases in parasitism are rare, but still significant. Numerous non-significant trends towards male biases have been found to be significant when pooled together (Poulin, 1996). Sex biases are more likely to be detected in experimental settings (vs. naturally

infected individuals) and in reproductively active individuals (Schalk & Forbes, 1997). Sex-biased parasitism is generally male-oriented. This is seen in reviews by Moore & Wilson (2002), Zuk & McKean (1996) and Poulin (1996), where the effect was strongest in mammalian and avian hosts, compared to fish. There are some examples of female-oriented sex bias, including breeding adult birds (McCurdy et al., 1998) and the black rhino (Hudson et al., 2002, as cited in Moore & Wilson, 2002), but these are the exception to the rule.

Numerous hypotheses have been propounded to explain the difference in parasitism between sexes (Zuk & McKean, 1996). Physiological differences between the sexes are often cited as potential causes for sex bias in parasitism and immunity in general. The heterogametic hypothesis suggests that males are inherently more prone to immune deficiencies (and hence parasitism) because they lack two copies of genes carried on the X chromosome. If this were true, then the trend would be reversed in birds, where females are the heterogametic sex. Zuk & McKean (1996) in their review found no sex bias in parasitism in birds, but a more recent study of breeding birds (McCurdy et al., 1998) found a female bias.

Immunological differences between the sexes in vertebrates are often postulated to be the result of sex hormones, particularly testosterone. Testosterone simultaneously increases reproductive behaviour and decreases aspects of immune function. In experimental settings, sex biases disappeared when males were castrated, and reappeared when castrated males were given hormone supplements (Zuk & McKean, 1996, and references therein). This aspect of gender-based differences in immunity has been often studied and explains sex biases very well, at least in vertebrates, but does not preclude

other physiological causes. The effects of estrogens are less clear, and other, non-sex hormones and physiological differences could also play a role.

Another hypothesis regarding sex biases in immunity that has received much attention in the literature is the idea of differential exposure. One sex or the other could, because of behavioural differences, be exposing themselves to infection more often than the other, resulting in higher parasite loads. Gregarines, for example, are contracted by ingestion of contaminated food, so differences in feeding habits of the sexes could result in differential infection. Likewise, males and females may be making different life-history trade-offs at the cost of immunity (Zuk & Stoehr, 2002). These two hypotheses are interrelated. A male, for example, may invest a great deal of time and energy in territory defense and high reproductive output, at the cost of investing in immune function. This may also potentially expose him to higher rates of parasitism as he travels more, is injured in territorial fights, and encounters more conspecifics than a female. Conversely, a female must invest more energy in the production of each offspring than a male, and may compensate for this by investing less in immunity, or may experience higher exposure to parasites while foraging for food. The exact nature of such tradeoffs is not yet clear. They seem to depend on many factors, and possibly are not consistent across species or taxa. Physiological and life-history trade-off explanations are not mutually exclusive, and together do a good job of explaining many cases of sex-biased parasitism, at least in vertebrates (Zuk & McKean, 1996).

The effect of host sex on parasitism has not been as well studied in invertebrates (Zuk & Stoehr, 2002), although trends towards male biases may be expected based on the same life-history principles proposed for vertebrates (Zuk & McKean 1996). However,

there is no hormonal analog to testosterone in invertebrates that would simultaneously promote reproduction and inhibit immunity (Wedekind & Jakobsen, 1998). It is difficult to make predictions based on physiological differences between the sexes in invertebrates for this reason.

It is far from clear whether there is a trend in sex biases in invertebrates. Some observations and experimental work support the prediction of male-oriented bias. For example, Wedekind & Jakobsen 1998) found a strong male-oriented bias in parasitism of a copepod host by helminth parasites under experimental conditions. Likewise, male dragonflies suffer higher prevalence and intensity of mite parasitism than females because of different habitat use patterns (Lajeunesse et al., 2004). Conversely, female-oriented bias has been reported for various species respectively of coenagrionid and lestid damselflies carrying gregarines (Hecker et al., 2002), and ectoparasitic mites (Robb & Forbes, 2005). Female *Lestes viridis* damselflies in the wild were found to have a higher expression of phenoloxidase (a component of the insect immune system) than males (Rolff, 2001), but whether this is a result of higher infection rates or a way to prevent them is unclear.

Still more studies reveal mixed results. Smith, in his 1988 review of the literature on parasitic water mites, cites six studies demonstrating sex bias in nature, three demonstrating no sex bias in the lab, and seven in which there was inconsistent bias or inconclusive results. A review of parasitism in arthropod hosts found, among several non-significant results, six examples of male-oriented bias and three examples of female-oriented bias, but no overall effect (Sheridan et al., 2000). Two *Coenagrion* spp. damselflies had equal abundance of parasitic mites under natural conditions (Rolff,

2000), and in the damselfly *Enallagma ebrium*, sex has been shown to be unrelated to mite load over five years of study (Forbes & Baker, 1990, 1991).

The nature of the difference in parasitism – if it exists – between the sexes in invertebrates has yet to be as clearly elucidated as it is in vertebrates. Likewise, it has yet to be shown what factors might account for the observed differences, or to explain why there is no difference in some cases where it would be expected. Much of the research to date has been done on odonates, primarily damselflies carrying *Arrenurus* spp. mites. Research on host-parasite relationships from other taxa is needed before generalizations can be made. It is particularly important that sex biases in parasitism may, in turn, influence dispersal of parasites if the dispersing sex is more likely to be parasitized. In addition, if the sexes vary in timing of emergence or activity it could lead to imbalance in samples of studies examining parasitism for some other reason.

The ERH predicts that invasive species will experience less parasitism in their introduced range; thus the length of time a host has been at a given site should affect parasitism. The longer as species has been present in its novel range, the greater chance that native enemies will have adapted or learned to exploit it. As an invasive species expands its introduced range, it will also encounter more enemy species. A positive correlation between time since introduction and impact of enemies would be expected. In addition, host population density, host age and relative date may influence parasitism. Population density can impact the spread of infectious agents and the proportion of individuals within a population who are infected. Higher-density populations are expected to experience proportionately higher rates of infection. This is generally true for parasitic as well as pathogenic infection (Wilson et al., 2002), although in some cases

no effect is seen (Matsumoto et al., 2004), or the reverse relationship is true (Rohlfs & Hoffmeister, 2004). Nevertheless, host population density can and often does have a significant impact on rates of parasitism and infection.

Host age also may be expected to influence parasitism, as older hosts may have time to accumulate more parasites, or parasites may be stage specific. Relative date within a season may affect parasitism, as parasite population levels increase along with host population levels (e.g. Hecker, 1999).

Galerucella calmariensis (Coleoptera: Chrysomelidae) was introduced to North America from Europe in the early 1990's for biological control of the invasive wetland plant purple loosestrife (*Lythrum salicaria* L.) (McAvoy & Kok, 2004, Landis et al. 2003, Lindgren, 2003). Purple loosestrife is a perennial wetland plant native to Europe, introduced to North America in the 1800's with discarded ship ballast (Thompson et al.., 1987, as cited in McAvoy et al., 1997). It is highly invasive and often forms dense monocultures in wetlands, to the exclusion of ecologically important native species (Blossey et al., 2001, McAvoy et al.., 1997).

A survey of 120 insects feeding on purple loosestrife in Europe eventually resulted in the selection of two Chrysomelid species, *G. calmariensis* and *G. pusilla* for release as potential biocontrols in North America (Malecki et al., 1993). In their native range, *Galerucella* spp. beetles can stunt *L. salicaria* plants and prevent flowering when present in high densities (Katovich et al., 2001). Both species were released in many sites in Canada and the United States in the early 1990's; however, *G. pusilla* has often failed to establish or persists at low levels compared to *G. calmariensis* (Dech & Nosko,

2002, Landis et al., 2003, McAvoy & Kok, 2004), possibly because of a reduced growth rate in low temperatures (McAvoy & Kok, 2004).

Below, I highlight what is known or expected of the natural history of *Galerucella calmariensis* in North America. *G. calmariensis* over winters in the adult stage. Adults emerge in the spring and feed and mate for approximately seven to ten days before beginning oviposition, which occurs throughout the season but with declining frequency. Eggs hatch after a few weeks and larvae develop though three instars over two to three weeks. Larvae then bury themselves in leaf litter at the base of the plant (or in aerenchymous root tissue if the plant is surrounded by water (Katovich et al., 2001)), emerging as teneral adults one to three weeks later (Denoth & Myers, 2005, Henne et al., 2005). After approximately one week, tenerals have assumed full adult colouration (pers. obs.). Some adults enter diapause and breed the following year, but some will breed and produce another generation in the same season (Kaufman & Landis, 2000). The number of generations that can be completed in a given season varies depending on temperature and food availability, and can be as few as one (Kaufman & Landis, 2000) or as many as three (Dech & Nosko, 2002) in various parts of Canada.

Larvae of *G. calmariensis* typically feed on the growing tips of purple loosestrife plants, where the risk of predation is less than on lower leaves (Sebolt & Landis, 2002). As ideal food at the apex of the plant is consumed, larvae will move downward seeking more food (Kaufman & Landis, 2000). Larvae feed by consuming the mesophyll and lower epidermal layer of leaves, leaving the upper epidermis intact and producing a 'window pane' effect (Katovich et al., 2001). Adult feeding produces a characteristic 'shot hole' damage pattern (Dech & Nosko, 2002), consisting of clearly defined holes

puncturing all layers of leaf, leaving surrounding tissue intact (Corrigan, 2004). Extensive feeding can result in complete destruction of the fleshy parts of the leaves on the host plant, known as skeletonizing or scorching (Corrigan, 2004).

Although adult *G. calmariensis* are capable of flight, they rarely use this method of locomotion. Flight is typically in response to adverse conditions (i.e., depletion of food resources) and usually covers only a brief distance (Grevstad & Herzig, 1997). Consequently, dispersal is very slow in this species (McAvoy et al., 1997, Dech & Nosko, 2002). Beetles will fly farther in search of food (up to approximately 20 m/day to a maximum of about 850 m between food patches – (Grevstad& Herzig, 1997)), and there is some indication that beetles will disperse more quickly downstream along rivers as adults may fall into the water and be carried some distance before being able to climb out again (Corrigan, 2004).

It has been argued that the 'planned invasions' that take place when biocontrol agents are introduced to control plants may be ideal models for studying invasion biology (Ehler, 1998). *Galerucella calmariensis*, although intentionally introduced and considered to be beneficial, can be considered an invasive species in ecological terms. Enemy release for weed-biocontrol agents is artificial, as released insects are first reared in the lab, (e.g., Hight et al., 1995), and are free of most if not all natural enemies upon release.

G. calmariensis meets just about every requirement for being labeled an invasive species. It is non-native, has established at sites of introduction and spread on its own from those sites, and causes significant changes in the ecosystem where it is established. It frequently attains such high densities that it exceeds its population carrying capacity

and defoliates entire stands of *L. salicaria*, which, according to Hairston et al. (1960) is characteristic of invasive insects. Were *L. salicaria* a native plant or one of economic importance, *G. calmariensis* would be considered harmful. Thus, while *G. calmariensis* is considered desirable, it may also be considered a model for invasive insects, and studying it may yield information that generalizes to other invasive species.

It is difficult to say to what degree *G. calmariensis* escaped its European enemies when it was introduced. It undoubtedly was released free of predators and parasitoids (Malecki et al, 1993). Enemies such as fungi and gregarines may not have been completely lost during the rearing procedures, as laboratory colonies are difficult to keep free of these more cryptic endophagous enemies. Collection of parental stock as larvae and pupation on florists foam rather than soil (Hight et al, 1995) make transfer of these enemies to North American unlikely, but the possibility cannot be concretely rejected. Nevertheless, as *G. calmariensis* spreads on its own, it could potentially undergo a sort of 'mini' enemy release from endophagous enemies, if beetles in recently colonized areas have fewer natural enemies than those from older sites. Furthermore, variation on this scale could give an idea of how time elapsed since introduction might bias more comprehensive studies one way or another or how local variability, host sex, or other factors might do the same.

The aim of this study was to explore the effects of the confounding factors mentioned above on parasitism in a model invasive species, *G.* calmariensis, with specific attention given to sex-biased parasitism. Visual examination under magnification for both external and internal parasites, and a simple assay for culturing entomopathogenic fungi were the tools used to assess parasitism.

I also was interested in whether, after assessing the importance of the other factors, there was still significant variation in parasitism explained by the length of time a population had been present at a site. I expected that this effect would be the following: beetles at sites where they had been established for some time (old sites) would have higher parasitism rates than sites where beetles had dispersed to recently or very recently (younger sites). This expectation is based on the assumption that dispersing insects experience less parasitism than non-dispersing insects, as per the ERH. My research also has the potential to inform future studies on accumulation of native enemies by introduced species because I have shown that mites, fungi, and gregarines are not uncommon parasites in *G. calmariensis.*

Materials and Methods

Collection Sites

Collection sites included both original release sites from *G. calmariensis* release programs taking place in Ontario between 1992 and 1997, and sites that the beetles has spread into unassisted. Original release sites were selected from a list of sites surveyed for *L. salicaria* and two *Galerucella* spp. in 2004 (Corrigan, 2004). Colonized sites were selected based on likely spread patterns of *G. calmariensis* and anecdotal reports of the recent arrival of the beetle. Sites are scattered across Eastern Ontario, though some recently colonized sites are located in South-western Quebec. Sites were selected to be geographically separate from each other, while still easily accessible.

The following is a description of locations with historical releases over 10 years ago (1992-1994). The locations include sites at Numogate, Packenham, Glen Tay, Lombardy and Almonte.

Numogate, ON is a classic and much-studied release site. It was included in the original release program in Ontario, and has been surveyed many times in the intervening years. These surveys indicate that purple loosestrife is in a stable state of control (Corrigan, 2004). On June 30, 2005, 17 beetles (11 F: 6 M) were collected from a roadside wetland in Numogate. On July 7 a return trip yielded 127 (61 M: 66 F) recently emerged adult beetles from three different roadside wetlands (all wetlands were located within Numogate town limits, along HWY 15). Though the sample of teneral adults was high in specific microhabitats, overall density was low, and feeding damage to purple loosestrife plants indicated low population density on both visits (see below).

The other visits and collections are described below. On July 26, 120 (50 M: 70 F) adult beetles were collected from Packenham at the intersection of RR 29 and the Mississippi river. Feeding damage indicated high population density, though the density of purple loosestrife was relatively low (isolated plants rather than large stands). On July 11, 116 adults were collected from Glen Tay at the intersection of Glen Tay Rd. S and Jeb's Creek. Feeding damage was high and there were many stunted plants displaying the pincushion growth pattern. A return visit on July 19 yielded 46 beetles (16 M: 30 F), to replace those which had inadvertently died and could no longer be dissected. Population density had decreased notably. There was evidence of high feeding damage in the past, but recent growth showed only medium density. On July 11, 122 adults were collected from a roadside wetland located at the intersection of HWY 15 and the Regional Road 1. Feeding damage was high, and plants were scattered, stunted, and not blooming. Finally, 16 adults (8 M: 12 F) were collected along a stream intersecting Wolf Rd. and downstream towards the Almonte cemetery on June 29. Feeding damage was low, and extensive searching did not yield more beetles.

The following is a description of locations with historical releases < 10 years prior to sampling (1996-2000). The locations include sites at Carleton University, Baxter Conservation Area, Kemptville, Pembroke and Arnprior.

From June 3 to June 20, a total of 126 adults were collected from the field research compound behind the Nesbitt Biology Building on the Carleton University Campus. Feeding damage at this site was of medium intensity. On June 24, 41 adults (19 M: 22 F) were collected from the Baxter conservation area along the Rideau river.

Feeding damage was high. All accessible waterfront habitat in the area was searched. A return trip on June 28 yielded 17 beetles.

In comparison, 9 beetles were collected from wetland along the Rideau River in Kemptville, near the intersection of HWY 43 and Rideau River Rd., beside the Kemptville water treatment facility on June 24. Feeding damage was low, and density of purple loosestrife plants was low. A return trip on June 28 included a broader search area and 54 more beetles were collected.

On July 6, 131 adults (60 M: 71 F) were collected in Pembroke, along the railway line and the Ottawa River riverfront. Feeding damage was low along rail lines and few beetles were found there. Feeding damage was high along the riverfront. On July 6 eighty adults (28 M: 52 F) were collected from two sites in Arnprior. The first site was a swimming area at the end of Sleepy Pines St. in east Arnprior. Feeding damage here was high, though there were few plants. The second site was in central Arnprior along the Ottawa River north of HWY 17. Feeding damage here was medium.

The following is a description of locations that were colonized very recently (2004-2005). The locations include sites at Petrie Island, Rockland, Chelsea, and Lac des Feés

On July 21, 114 adults were collected from a wetland along the causeway leading to Petrie Island from HWY 174. Surveys in 2004 found only one beetle at this site (Cappuccino, pers. communication). Feeding damage was low, and dense stands of purple loosestrife were present. On July 21, 30 adults were collected from roadside ditches along HWY 174 located from four to five km east of Petrie Island. Feeding damage was low. On July 28, forty-five adults (15 M: 30 F) were collected from Chelsea,

QC. Individuals were found around the smaller of two ponds located south of the Chelsea Pioneer Cemetery on Old Chelsea Rd. There was evidence of high density in the past, but feeding damage on recent growth was low. On July 28 fifty-one adults (26 M: 25 F) were collected from the edges of Lac des Feés in Hull, QC. Purple loosestrife was abundant at the site, and though there was evidence of high population density in the past, feeding damage was low on recent growth.

I also sampled at three additional sites that are not included in the data set. The original release site in Carp was visited on June 29. The intersection of the Carp river with HWY 49 (also called Hwy 44 and Carp Rd), where the original release was done in 1994, was inspected, but no purple loosestrife plants (and therefore no beetles) were found.The original release site in Antrim could not be located when the site was visited on August 6. Isolated clumps of purple loosestrife were present in roadside ditches, but insufficient beetles were found to warrant inclusion in the data set. The recently-colonized area along HWY 511 from Perth to Calabogie was explored on August 6. A single adult beetle was found in a wetland at the intersection of HWY 511 and County Rd. 15 just south of the boundary for Lanark County. A large number of late – instar larvae were present at this site and feeding damage was high. A return trip on August 9 yielded 5 adults beetles, and the number of larvae had decreased markedly. A third trip on August 15 yielded a single beetle and no larvae were found. Plants were almost completely skeletized at this point, bearing inflorescences but few or no green leaves. This suggests that the local beetle population has recently exploded, and then crashed when the food supply was exhausted, and the remaining larvae had failed to complete development.

Geographical distribution of collection sites is shown in Figure 1 and a summary of the beetles collected by site, sex, beetle age and population density is shown in Table 1.

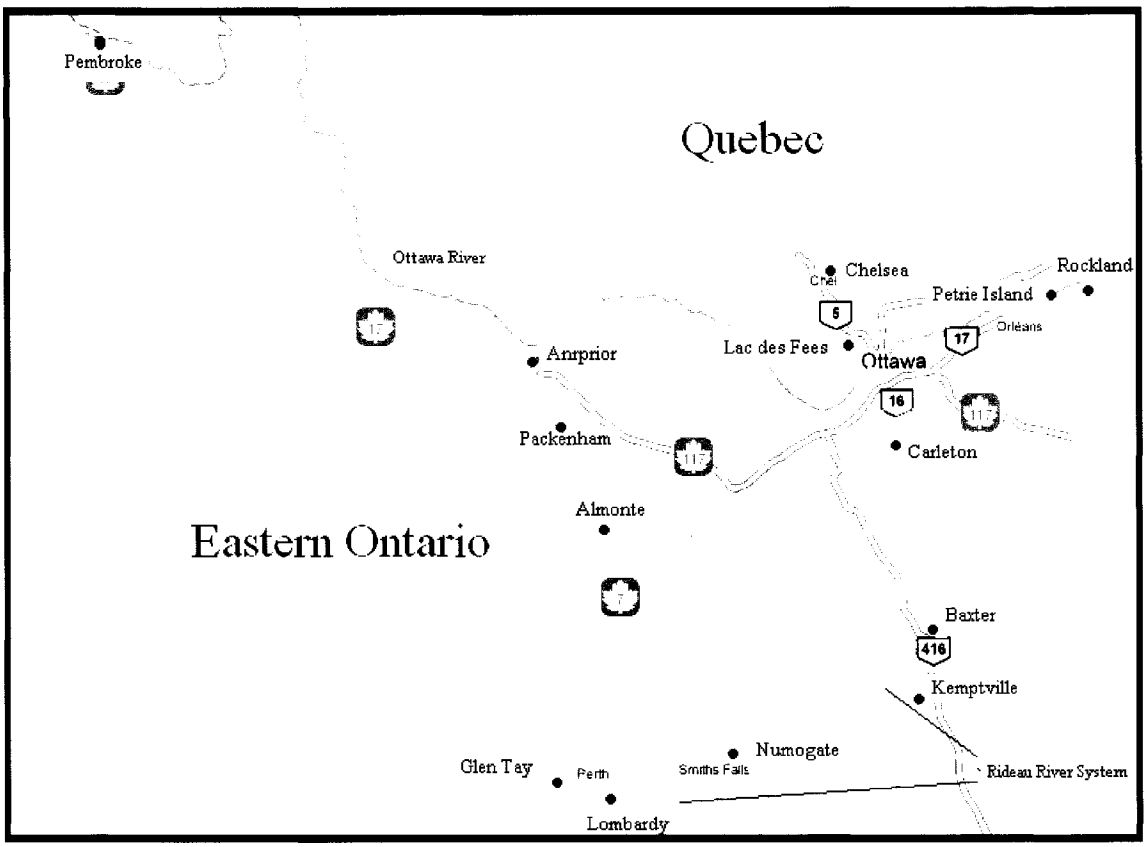

Figure 1: Collection sites in eastern Ontario and southern Quebec. Collection sites are marked with a red dot.

Table 1: Summary of beetles collected at fourteen sites, by sex, age and population density.

Site	Total # of Beetles	# Males	# Females	Beetle Age	Population Density
Almonte	21	9	12	Over wintered	Low
Anrprior	80	29	51	Teneral	High
Baxter	40	18	22	Over wintered	Med
Carleton	117	54	63	Over wintered	Med
Chelsea	37	15	22	Adult	Low
Glen Tay	104	46	58	Adult	Med
Kemptville	63	25	38	Over wintered	Low
Lac des Fées	50	22	38	Adult	Low
Lombardy	104	40	64	Teneral	High
Numogate	77	34	43	Teneral	High
Packenham	112	61	61	Adult	Med
Pembroke	115	54	61	Teneral	High
Petrie Island	99	52	47	Adult	Low
Rockland	30	9	21	Adult	Low

Sampling of Beetles

Adult insects were located by careful visual inspection of plants, and collected by hand. Because *Galerucella* spp. beetles drop from their host plants when disturbed, this had to be done with care to disturb the plants as little as possible. Beetles were placed in glass jars with some plant material. Beetles were kept separate according to collection site. After being collected, beetles were taken to the lab, where they were examined and separated according to sex.

Population Density Estimates

Population density was estimated by observing feeding damage on host plants. This was deemed to be the most effective means of estimating population density because the apparent density of adult beetles is influenced highly by weather conditions and the degree to which the habitat is disturbed. On warm, sunny days adults climb to the tops of plants to bask and look for mates, whereas on cloudy or cool days they remain hidden. Under calm conditions, beetles sense disturbances to the brush more easily and drop from their host plants to the ground when the plant is disturbed. The effects of wind obscure disturbances caused by moving through the brush, and beetles are less likely to drop under windy conditions (pers. obs.). For these reasons, density estimates based on number of beetles found per unit of time or area were deemed unreliable.

Feeding damage remains more or less static over time, and damage caused by adult beetles is distinct from that caused by larvae. Because no native North American herbivores exert a significant impact on purple loosestrife (Malecki et al., 1993), all feeding damage can be attributed to *Galerucella* spp. feeding.

For purposes of this study, feeding damage was rated categorically as low, medium or high. In areas with low levels of feeding damage, the majority of plants bear little or no damage that is visible from a distance. Close inspection reveals isolated damage on a few leaves/plants. In areas of medium damage, the majority of plants display some obvious damage, but appear to be growing vigorously despite feeding. Isolated plants may be stunted or skeletized. Grevstad & Herzig (1997) consider a density of 1 to 4 beetles per stem of loosestrife to be a moderate population density. This falls within the range observed on plants with 'medium' feeding damage, as 'low' damage plants often had no beetles, and 'high' damage plants contained upwards of 10 insects per stem. In areas of high damage, the majority of plants appear stunted and discoloured, and may be completely defoliated. In areas subjected to high *Galerucella* spp. density for many years, plants form small, dense growths called 'pincushions' and often fail to flower (Corrigan, 2004).

Using feeding damage as an estimate of population density relates density to food supply; something more biologically relevant than area alone, as the density of food plants varies from site to site and over time. By the same token, however, estimates based on feeding damage can be misleading as to the number of individuals in a given habitat patch. A site with a high density of purple loosestrife may contain a large number of beetles, but their density per plant may be low. This is important to remember when considering the effects of density. Feeding damage was generally consistent throughout a habitat, and initial density estimates were made based on the first clump of plants where beetles were located. In a few cases, distribution of beetles was highly patchy and in this

case density estimates were taken in the patches where the most beetles were collected, i.e. in the higher density areas.

Sexing

Initially, beetles were sexed behaviourally. During breeding, male *Galerucella* spp. mount the female and remain with her while she forages. Whenever possible, breeding pairs were collected from the field, and the male and female separated in the lab. When breeding pairs were not abundant, all adult beetles were kept together and copulations were observed. Male *Galerucella* are smaller than females, which made distinguishing between individuals of breeding pairs easier.

A few weeks into the study, I learned how to sex individual beetles based on morphology rather than behaviour. Male *G. calmariensis* possess a black claw known as the tibial spur located at the distal end of the meso- and metatibia, approximately 60 nm in length that is only visible under ~ 30 x magnification (Cosse, 2004).

After being sexed, beetles were allocated either for dissection or for surface sterilization and fungal assay.

Species Identification

Although *Galerucella calmariensis* and *Galerucella pusilla* have different phenotypes, the differences are slight and intraspecific variation in colouration means that they often are indistinguishable in the field. *G. calmariensis* is far more common than *G. pusilla*, which seems to have declined since introduction (Corrigan, 2004, Dech & Nosko, 2002). In the lab, the two species can be distinguished by examination under a dissection scope.

In *G. pusilla*, the 11th abdominal segment is light coloured, in *G. calmariensis* all the abdominal segments are the same colour (Manguin et al., 1993). A subset of individuals from a variety of sites (250 individuals) were examined under a dissection scope, and no specimens of *G. pusilla* were discovered. Although this does not preclude the possibility that a few individuals of this species were collected and processed, it indicates that all individuals were most likely *G. calmariensis*.

Dissection

A pilot study using *G. calmariensis* adults collected from Carleton University revealed that while macroparasites of this host are rare, infection by eugregarine gut parasites is nearly ubiquitous. Gregarines (Apicomplexa: Eugregarinida) are common gut parasites of many orders of insect, ranging in impact from negligible to lethal. They are generally found attached to the midgut endothelium (Siva-Jothy & Plaistow, 1999).

Beetles allocated for dissection were examined under a dissecting scope at 10 x magnification for external parasites. They were then euthanized by decapitation and dissected to check for internal parasites. The digestive tract was removed by gently pulling while decapitating the insect and placed in sterile Ringer's insect saline (0.13 M NaCl, 0.005 M KCl and 0.003 M CaCl in dH_20). The body cavity was opened using insect pins and checked for parasites. The digestive tract was then dissected under a scope at 30x magnification using size 00 insect pins to check for eugregarine parasites, which were counted.

Fungal Pathogen Assays

Beetles allocated to fungal pathogen assays were first surface sterilized using a procedure adapted from S. Cremer (pers. commun.). This method, and variants of it, have been used in many studies looking for fungal pathogens of insects (e.g. Bidodochka et al, 1998). Individuals were immersed briefly in 70% ethanol and agitated to aid wetting, then rinsed in distilled water and immersed in 1% NaClO solution for at least one minute before being rinsed in three changes of distilled water. Individual insects were then placed in the center of a 9 cm sterile Petri plate lid lined with moist, sterile filter paper (Whatman #42). Plates were kept in the dark at room temperature and watered with distilled water every 1-2 days for 20 days, at which point they were examined for fungal growth. By placing the filter paper in the lid of the Petri dish and stacking the plates in an inverted position, it was possible to water the plates without opening them by spraying the sides of the plates and allowing water to run down into the lid.

Fungi were examined under a dissecting scope at 30-40x magnification and identified at least to the genus level based on the morphology of the conidia (Bidochka et al., 1998). Fungi proved difficult to photograph in sufficient detail, so hand-drawn representations of the different species were prepared (Figure 2).

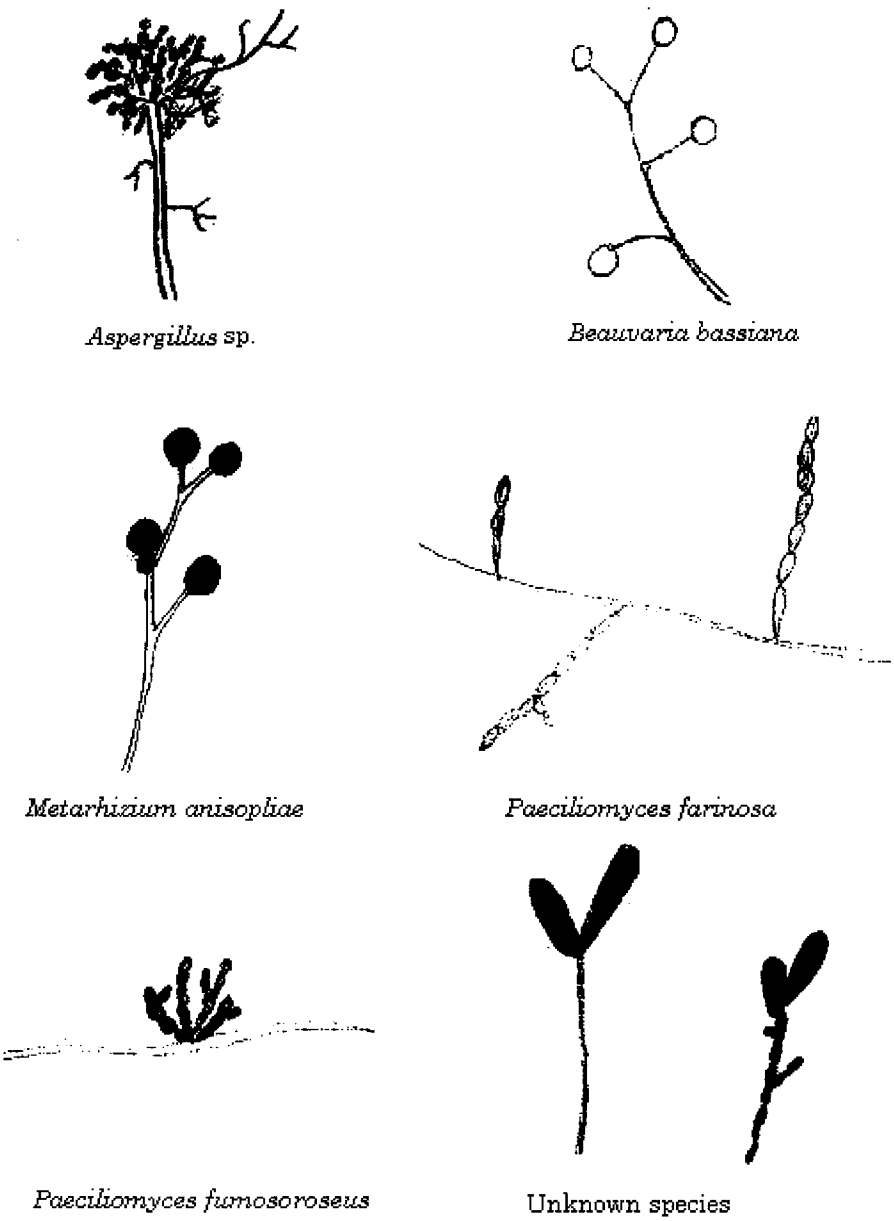

Figure 2: Fruiting bodies of six fungal species isolated from *G. calmariensis* .

Analysis

All data analysis was done using the statistical programs SPSS version 13 and R version 2.1.1. Where relevant, data were checked for normality and homoscedasticity using Shapiro-Wilks, Kolmorogorov-Smirnov, and F-tests. If these assumptions were violated, data were transformed by taking the square root. This was sufficient to make data conform to assumptions in all cases.

Mite and nematode data were analysed separately, using Fisher's exact test because of small sample size. Data from sites where these enemies were not recovered were excluded from each respective analysis.

Gregarine data violated the assumptions of parametric tests. The raw data were found to be heteroscedastic (F-test to compare variance: $F = 5.2$, $p=0.0079$) and female data were borderline non-normal (Shapiro-Wilks, $p= 0.067$, $W = 0.0878$). After a square-root transformation on all data, these problems were corrected. Non-normal data were generally skewed towards one end of their range, making the square-root transformation a good choice. Both male and female data met assumptions of normality (Shapiro-Wilks, $p= 0.37$, $W = 0.93$ (male), $p = 0.21$, $W = 0.91$ (female)), and homoscedasticity (F-test, $p=0.19$, $F= 2.19$, $df=12$). Average parasite loads for male and female hosts were paired by site to control for other factors which might influence parasite load, and the data were compared with a paired t-test.

A Chi-square test was done to compare bulk fungal infection rates between males and females. Data from each fungal species were examined separately to determine if one sex was more likely to be infected. In order to statistically control for other confounding variables, a logistic regression was performed for data from each fungal

species. The predictor variables were sex, beetle age, site age, relative date, and relative population density. The response variable was infection status, which could be positive or negative.

To address the ERH, macroparasite data were analysed using Fisher's exact test because of the small numbers of parasites recovered. Only data from old sites (from the original release programs) and recently colonised sites were used because of the 2 x 2 design requirements of Fisher's exact test.

The data on gregarine loads had significant issues with correlated variables that could not be adequately solved by statistical means. This precluded useful analysis by such means as ANOVA, as many variables would have to have been disregarded. For this reason, three pairs of old and recent collection sites that were sampled around the same time were selected for analysis. This controls for effect of relative date and beetle age. These site pairs were Packenham(old) – Chelsea(recent), Glen Tay – Lac des Feés, and Lombardy – Petrie Island.

Gregarine data from these pairs were square root transformed and each pair was analysed separately using a two-way ANOVA with sex as a fixed factor and site age as a random factor, using type III sums of squares.

Fungal infection data were not subject to the same problems with correlated variables that gregarine data had, according to SPSS version 13. There was an overall trend towards higher infection rates later in the season (an effect of relative date, $p = 0.017$), but this was mainly due to a significant trend in one of the species present (*Aspergillus* sp., $p = 0.021$, see figure 7) and was not significant in other relevant species.

The unknown species also showed a significant effect of date ($p = 0.013$) but was not included in the analysis because of small numbers of infected beetles.

Aspergillus sp., *B. bassiana*, and *P. farinosa* were selected for further analysis because of their large representation in the sample. *M. anisopliae* was also included, but the results should be viewed with caution because of its small sample size. *B. bassiana* and *P. farinosa* are of the most interest because relative date is not a significant predictor of prevalence in these species.

Individual logistic regressions using Type III sums of squares were performed on data from each of the aforementioned species. Sex, relative date, site age, beetle age and relative population density (beetles) were used as predictor variables

Results

I first address general results during the 2005 field season from fourteen sites in eastern Ontario and Quebec. Of the beetles collected at these, 512 (219 M: 293 F, representing thirteen sites) were dissected and examined for parasites, 537 (240 M: 297 F, representing fourteen sites) were assayed for fungal infection.

Three varieties of parasites were found during dissections. Ectoparasitic mites, most likely *Leptus* sp. (B.P. Smith, pers. comm.) were found at six collection sites. Nematode worms, thought to be mermithids because of their position in the body, were found at three sites. Eugregarine gut parasites (Figure 3) were found in virtually all beetles from all sites.

Gregarine parasites ranged in size from approximately 80 - 200μm. They were present almost exclusively in the foregut of the beetle (versus the mid or hindgut) and sometimes completely filled this region. Gregarines were usually attached anteriorly to the gut lining, but would detach and move around during dissection. Individuals consisted of two segments, though these would occasionally separate. Additionally, some individuals were seen undergoing fission, with one or two smaller segments attached to the posterior of a larger segment. In at least one host, oocysts were observed, indicating that the parasite undergoes sexual reproduction in this host.

Eugregarinida represents a diverse and understudied group. Reliable identification keys for gregarines have not yet been developed. With the exception of a few well-studied host-parasite systems involving gregarine parasites of commercially important insects, these parasites generally go unidentified, and it is not unusual in the

literature for them to be referred to simply as gregarines. From the uniform appearance of all the individual gregarines observed, it is likely that only one species was present.

Six fungal species were isolated. Four were identified to species level by visual inspection under a dissecting scope, a fifth was identified to the genus level, and one remains unidentified. The species recovered include: *Aspergillus* sp., *Beauvaria bassiana*, *Metarhizium anisopliae, Paeciliomyces farinosa, Paeciliomyces fumosoroseus*, and the unknown species. Identifications were based on descriptions and illustrations found in Kendrick (1992). Illustrations of the fruiting bodies of the fungal species are shown in Figure 2. The relative prevalence of each species is shown in Figure 4. All identified species are known to be entomopathogenic, although the genus *Aspergillus* is strictly an opportunistic pathogen, meaning that it does not attack healthy insects.

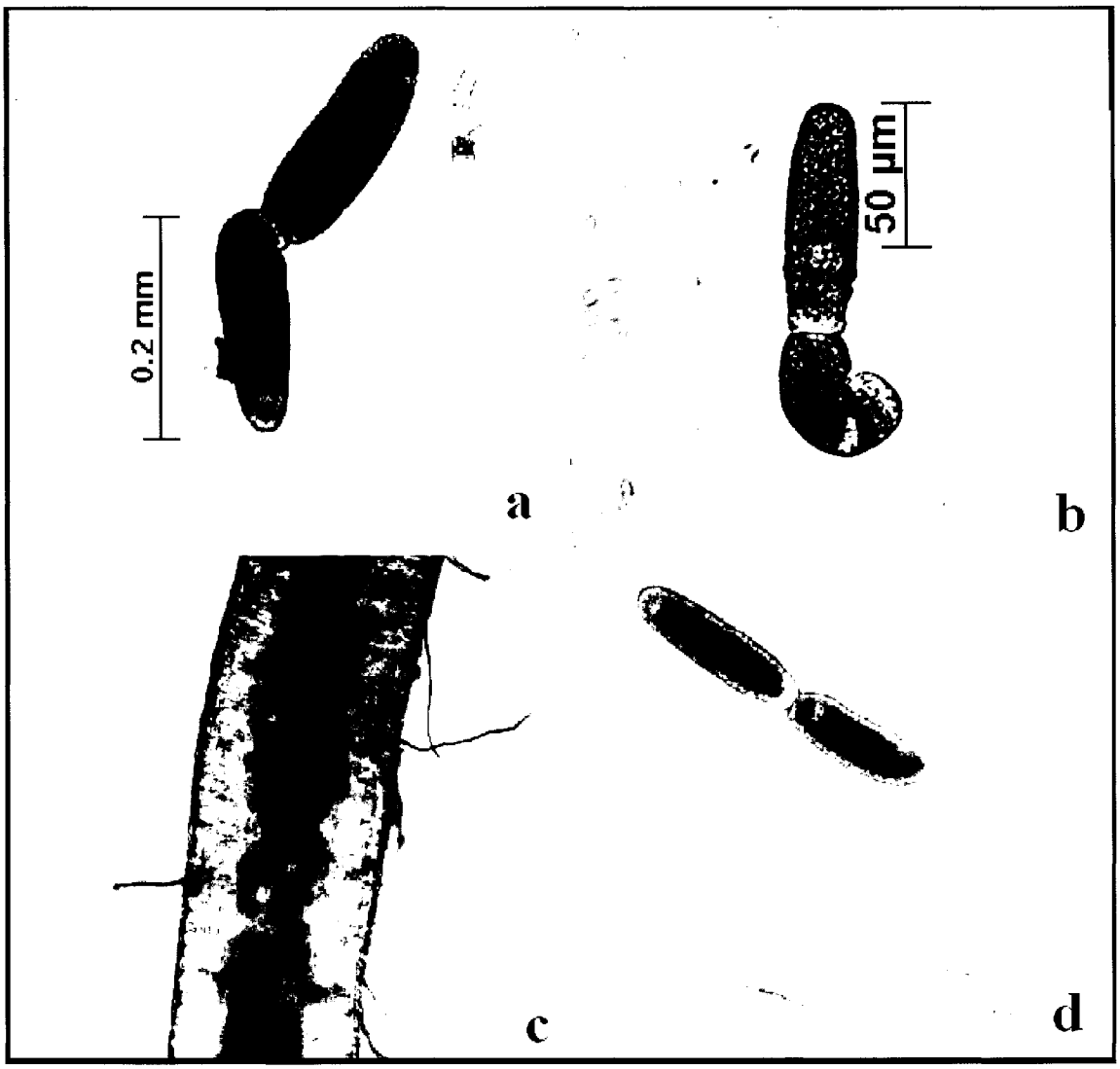

Figure 3: Eugregarine gut parasites; a) a representative large gregarine with scale bar, b) a representative small individual with scale bar, c) intact digestive tract of *G. calmariensis* with gregarines visible inside (dark bodies), d) large and small gregarines together.

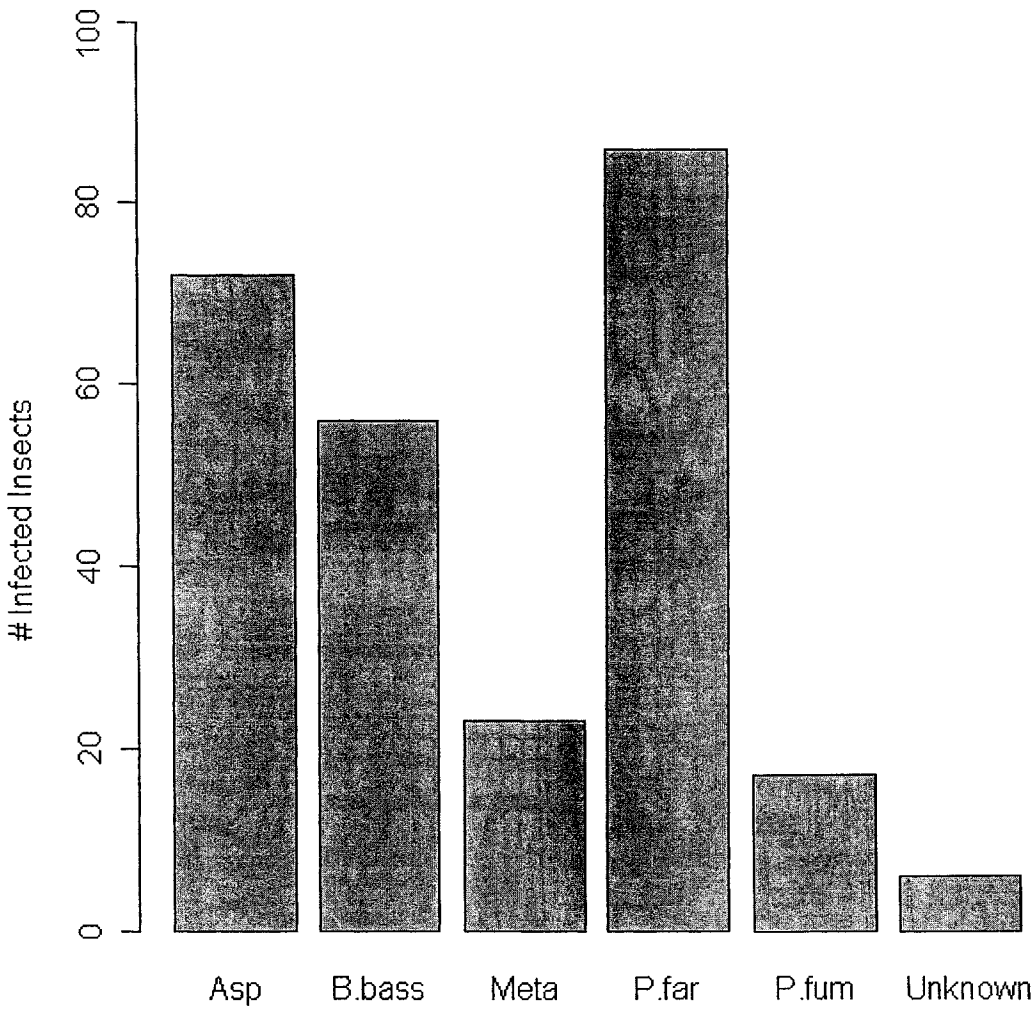

Figure 4: Relative abundance of six different entomopathogenic fungi cultured from *G. calmariensis* during the summer 2005 field season. Asp = *Aspergillus* sp., B. bass = *Beauvaria bassiana*, Meta = *Metarhizium anisopliae*, P. far = *Paeciliomyces farinose*, P. fum = *P. fumosoroseus*.

Enemy Release

Parasitism

Both nematodes and mites were more prevalent at more recently populated collection sites (Table 2).

Site age was found to be a highly significant predictor for both types of parasites ($p < 0.001$ for both). However, there was no way to control for other variables (beetle age, relative date, etc.) as was done in the sex bias analysis, so these results should be viewed with more caution.

Site age was a significant predictor of gregarine load in two of the three pairs selected (Table 3), with beetles from old sites carrying more gregarines. Sex was also a significant predictor for Glen Tay-Lac des Fées ($p = 0.012$). The selected data are displayed graphically (Figure 5).

Table 2: Infection rates for macroparasites across site ages. Total infection rate was obtained by dividing the number of beetles carrying each parasite by the total number of beetles collected. Infection rate at infested sites was obtained by dividing the number of beetles carrying each parasite by the total number of beetles collected from infested sites of that age bracket.

Site Age	**Recent (N=4)**	**Medium (N=5)**	**Old (N=5)**
# Sites With Mites	4	1	1
Total Infection Rate (Mites)	10%	0.7%	0.4%
Infection Rate at Infested Sites (Mites)	10%	5.8%	1.7%
# Sites With Nematodes	1	2	0
Total Infection Rate (Nematodes)	0.83%	4.1%	0.0%
Infection Rate at Infested Sites (Nematodes)	3.4%	9.0%	0.0%

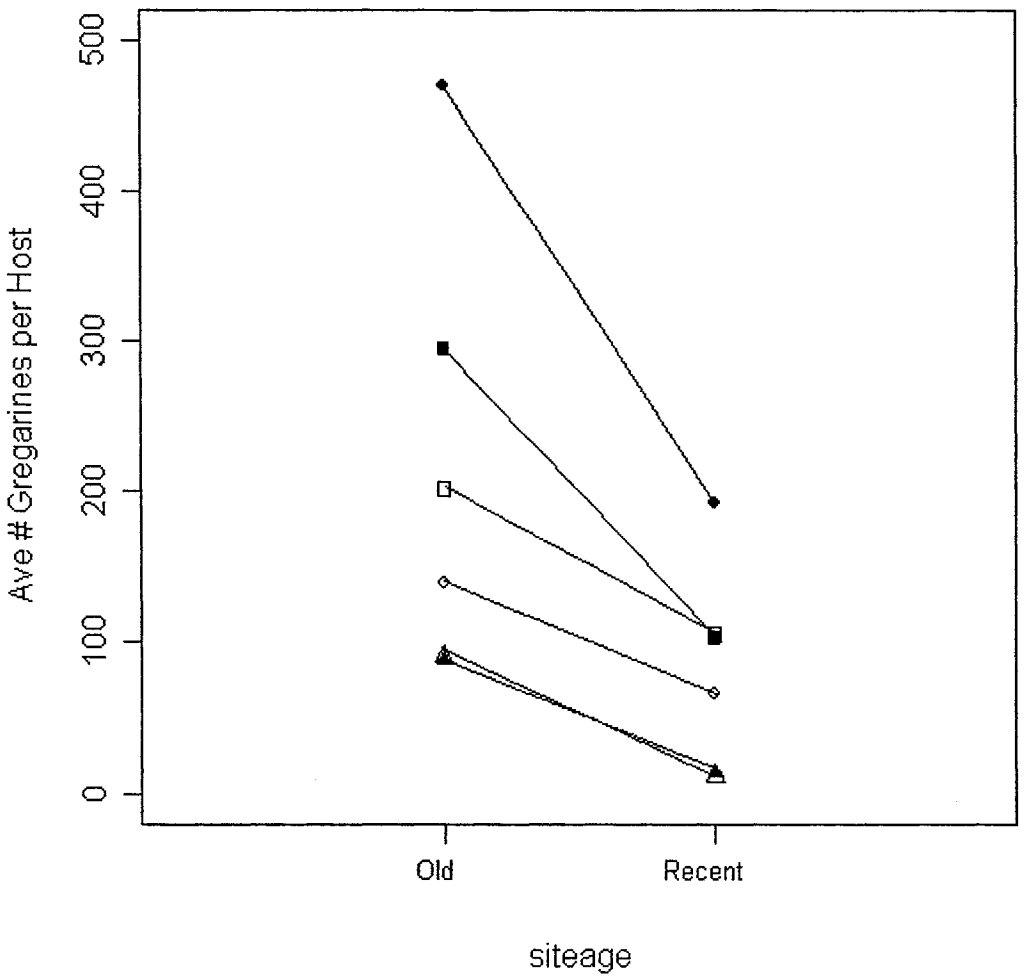

Figure 5: Untransformed gregarine counts for males and females from three pairs of old and recently colonised collection sites. Site pairs are: Packenham – Chelsea (squares), Glen Tay – Lac des Fées (circles), Lombardy – Petrie Island (triangles). Male averages are represented with open figures, female averages with dark figures.

Table 3: Gregarine load as a function of site age (ANOVA results). Significant values in bold.

Site Pair (Old-Recent)	**Ave. greg.** (Old)	**Ave. greg.** (New)	**Df**	**F-value**	**p-value**
Packenham-Chelsea	263.3	102.8	1,73	0.74	0.393
Glen Tay – Lac des Fées	304.2	172.2	1,71	6.7	**0.012**
Lombardy – Petrie Is.	89.6	12.9	1,100	26.9	**<0.001**

Fungi

Fungal infection is more prevalent at more recently colonized sites for all three fungal species where there is a significant effect. These species are *Aspergillus* sp. ($p= 0.035$), *P. farinosa* ($p = 0.009$), and *M. anisopliae* ($p= 0.011$). The relationship between site age and prevalence of fungal infection is shown in Figure 7.

Beetle age was a significant predictor for *B. bassiana*($p=0.002$), *P. farinosa* ($p=0.002$), and *M. anisopliae* ($p<0.001$). Fungal infection was more prevalent in older beetles for *P. farinosa* and *M. anisopliae* (Fig 8), but the reverse was true for *B. bassiana.* Relative date had a positive effect on fungal infection in Aspergillus sp. ($p=0.005$) and relative density had a positive effect on fungal infection in *M. anisopliae* ($p<0.001$). The results of the analysis are summarised in Table 4.

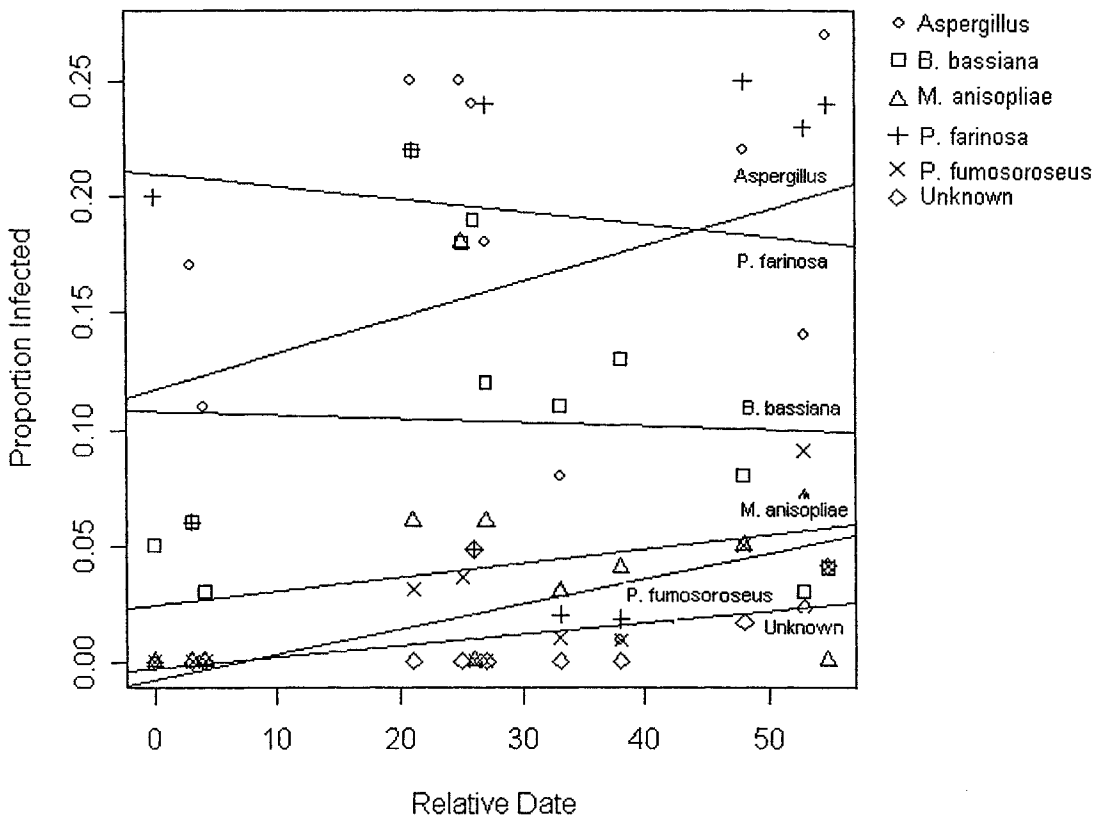

Figure 6: Proportion of *G. calmariensis* infected with individual fungal species as a function of relative date. The overall trend is significant ($p = 0.017$), but only *Aspergillus* sp. and the unknown species showed a significant effect of relative date ($p = 0.021$ and 0.013, respectively).

Table 4: Critical values for logistic regression analyses of fungal data. Statistically significant results are italicised.

Species	**Predictor**	**X^2**	**df**	**P**	**Pseudo R^2** (Naglekerke)
Aspergillus sp.	*Relative date*	*7.99*	*1*	*.005*	
	Sex	0.58	1	.445	
	Site Age	*4.46*	*1*	*.035*	
	Beetle Age	0.015	1	.903	
	Relative Density	0.08	1	.782	
					0.14
Beauvaria bassiana	Relative date	2.23	1	.135	
	Sex	1.41	1	.235	
	Site Age	1.06	1	.745	
	Beetle Age	*9.3*	*1*	*.002*	
	Relative Density	0.161	1	.689	
					0.054
P. farinosa	Relative date	1.21	1	0.27	
	Sex	2.67	1	0.103	
	Site Age	*6.75*	*1*	*0.009*	

	Beetle Age	*9.49*	*1*	*0.002*	
	Relative Density	0.11	1	.74	
					0.17
*M. anisopliae**	Relative date	1.79	1	.181	
	Sex	.934	1	.334	
	Site Age	*6.51*	*1*	*0.011*	
	Beetle Age	*13.5*	*1*	*<0.001*	
	Relative Density	*18.8*	*1*	*<0.001*	
					0.19

* Encountered warning message while analysing, due to small sample size. SPSS v. 13 cautions that results may not be valid.

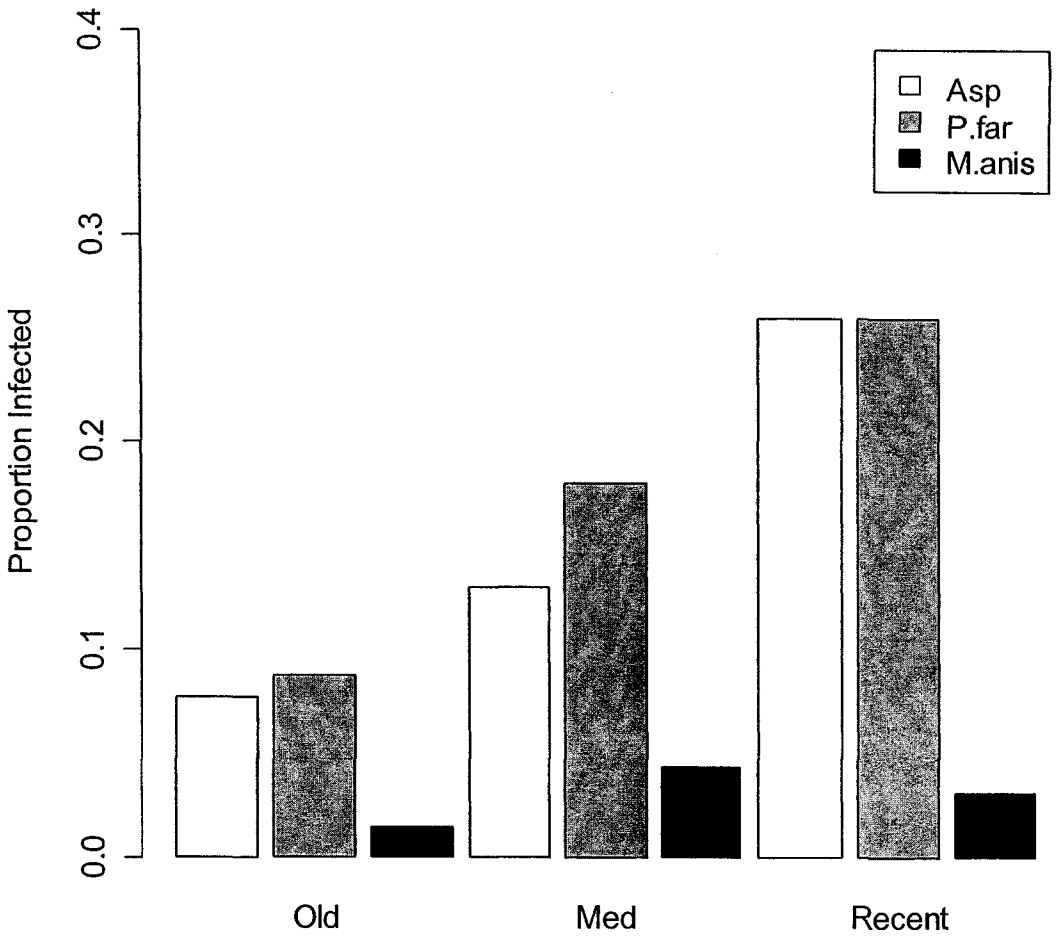

Figure 7: Proportion of *G. calmariensis* individuals infected with three fungal species at old, medium and recently colonized collection sites. Effect of site age is significant for each species (see Table 4).

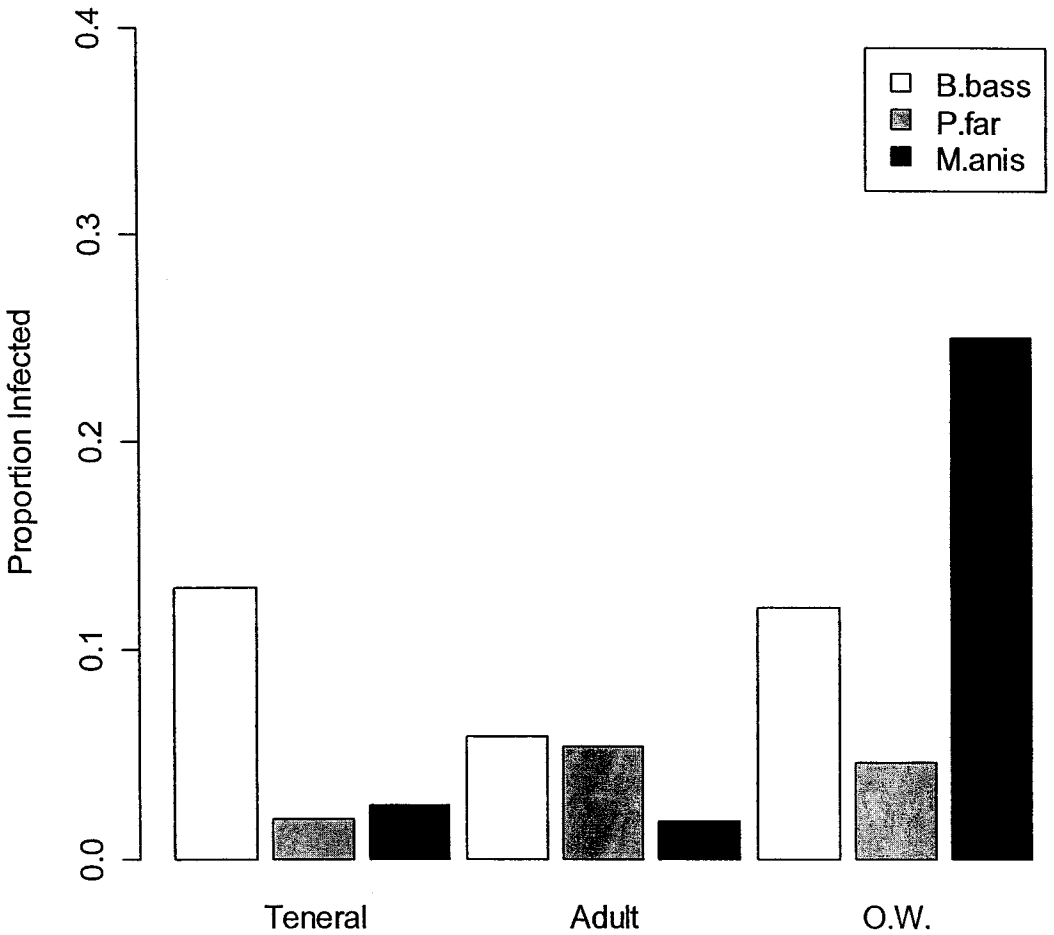

Figure 8: Proportion of *G. calmariensis* infected with three fungal species, according to age class. All relationships are statistically significant (see Table 4). OW = overwintered, the oldest beetles collected.

Sex Bias

Parasitism

Mites and nematodes were present in very small numbers, and data on these macroparasites were analyzed using Fisher's exact test. Mites and nematodes were tested separately. There was a significant female-oriented bias in infection by both mites and nematodes, when only data from sites where these enemies were observed were considered ($p<0.001$ for both, Fisher's exact test (two sided)). When the whole sample was considered, these effects disappeared because of the large number of zeros in the dataset.

Although there was female-oriented sex bias when prevalence of either parasite is considered, the intensity of infection was not significantly different between the sexes, even when only data from sites where these enemies were observed was included in the analysis ($p=0.9963$, Kolmorgorov-Smirnov test, K-S, $p = 0.4413$).

Gregarine parasites were present in large numbers. Males across all sites carried an average of 110.1 gregarines, females an average of 204.2 (Figure 9). The difference was found to be significant ($p = 0.0085$, $t = 3.14$, $df=12$). Parasitism is consistently higher in female *G. calmariensis.*

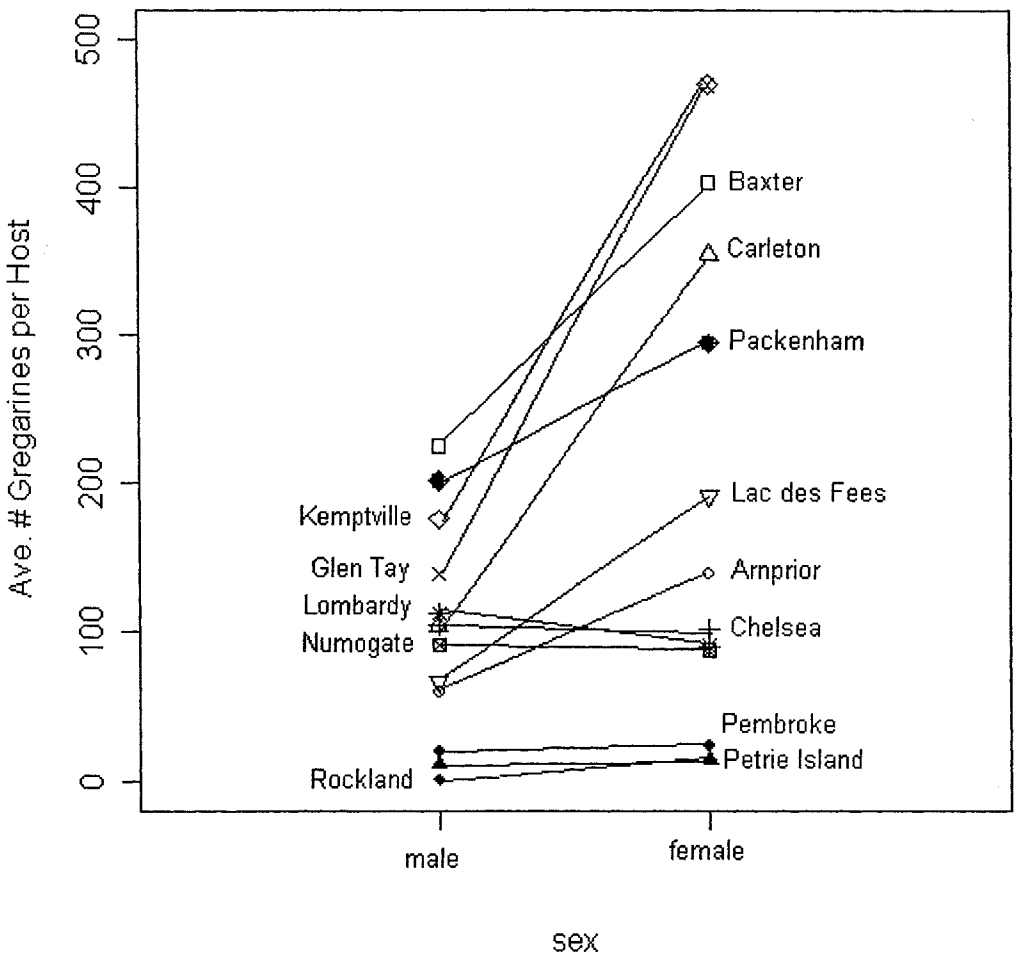

Figure 9: Average gregarine load of male and female *G. calmariensis* across thirteen sites. Females carry an average of 204.1 gregarines, males 110.1.

Fungi

Males experienced slightly higher fungal infection rates than females ($p=0.04$, $df=1$, $X^2=4.12$). Overall, 48.3% of males and 39.7% of females were infected with at least one fungal species. Approximately 5% of the sample was infected with two fungal species concurrently.

There was no significant effect of sex for any of the species considered. *P. farinosa* showed a trend towards male bias (Table 5; $X^2 = 2.67$, $p = 0.10$).

Table 5: Critical values for a logistic regression examining infection status of *G. calmariensis* for six fungal species, as a function of host sex. Other variables were controlled statistically.

Fungal Species	**X^2 value**	**p-value**
Aspergillus sp.	0.58	0.44
B. bassiana	1.36	0.24
M. anisopliae	0.95	0.33
P. farinosa	2.67	0.10
P. fumosoroseus	0.62	0.43
Unknown species	0.20	0.65

Discussion

As indicated, *Galerucella calmariensis* is an introduced biocontrol beetle native to Europe. It has been argued (Ehler, 1998) that biocontrol agents make ideal organisms for examining the phenomenon of enemy release, as they are generally released only after quarantine procedures to eliminate natural enemies. Furthermore, as a biocontrol agent spreads within its introduced range, it may undergo a sort of 'mini-release' from enemies. Studying the variation in natural enemies of *G. calmariensis* across a wide portion of its introduced range can offer insights into factors that may affect the enemy release process.

Studies of enemy release often fail to consider time since introduction of the host species, and when they do the timescale is frequently large (50 years or greater). Timescales are often drawn from early studies in the field, which were based largely on educated guesses. Whether shorter timescales make a difference is rarely considered, and the same can be said of other potentially confounding factors. By sampling across a broad area of the introduced range of *G. calmariensis*, and across an entire field season, factors such as beetle age, time of year, beetle population density, and host sex can be taken into consideration.

Host sex is an important factor contributing variation to parasitism. Bias towards or sex or the other is detected in approximately ten percent of studies. How sex influences immunity in general and parasitism in particular are subjects that have received much attention in the literature.

G. calmariensis beetles released in Ontario came from laboratory reared stock. In order to exclude parasitoid wasps that make use of adults and older larvae as hosts, parental stock was collected in Germany as 3rd instar larvae, which then completed their development in the lab (Hight et al., 1995). This treatment would also effectively exclude many other natural enemies. Hight et al. (1995) make no mention of how emerging adults were fed except to say on "fresh" food in rearing cages. Newly emerged adults are likely free of gregarines. This is true in damselflies (Siva-Jothy & Plaistow, 1999), and beetles collected shortly after emergence had few to no gregarine parasites (pers. obs), though in the crane fly *Tipula paludosa*, all larval stages carry gregarines in the gut and gregarines do not adhere to the eggs (Er & Gökçe, 2005). Gregarines are contracted through feeding on leaf tissue that has been contaminated with fecal matter from other beetles. Without knowing the source of the food plants or housing conditions (solitary or in groups) of the adults reared by Hight et al., (1995), it is impossible to know whether gregarine parasites could have been transferred from the native range. Furthermore, it is then impossible to know whether the gregarines found in this study represent host expansion of a native species, or co-introduction of a European species along with the beetle.

Entomopathogenic fungi are generally disseminated through spores in the soil (Bidochka et al., 1998): insects are infected when larvae enter the soil to pupate. Because the parental stock of *G. calmariensis* underwent pupation in florists' foam instead of soil (Hight et al., 1995), it is unlikely that they were contaminated with fungi. Furthermore, all insects shipped to North America were held in quarantine approx. 7 days and only healthy individuals were shipped to release and breeding sites. Although this does not

prove with certainty that no fungi-infected individuals were released in North America (fungi are often opportunistic pathogens and infected individuals may not have displayed symptoms during quarantine), it is still likely that this is the case. Appendix 2 contains more information about the natural history of parasite species in this study.

Both nematode worms and mites were more abundant at more recently colonized sites. This finding would go against predictions of the ERH. It would seem to provide at least anecdotal evidence for the biotic resistance hypothesis, except that *G. calmariensis* is a species which is spreading and invading new habitat. It is apparently not being limited by natural enemies to any great extent, although it is impossible to say this conclusively without empirical tests. It must be considered that this comparison is not a valid test of either hypothesis because there are no comparisons to native congeneric species or to *G. calmariensis* in its native range. It may, however, inform the issue. *G. calmariensis* spread hardly at all in the first few years after introduction, and perhaps parasitism by *Leptus* spp. mites and nematodes is partly responsible for this and the slow spread that the beetle currently exhibits. Parasites are considered to be the agent responsible for inhibiting the spread of the tussock moth, *Origya vestusta*, another invasive insect with weak dispersal capabilities (Hastings et al, 1997), although the dynamics may be different for this host-parasite relationship.

Because it is known that parasites can sometimes manipulate the behaviour of their hosts in a way that increases the parasite's fitness, a literature search was performed to determine if there is any indication that either nematodes or mites can induce dispersal in insect hosts. If this occurred, it would explain why these parasites are more common

at more recently colonized sites, as populations at these sites would have been established by infected individuals. No examples of either class of parasite causing dispersal in insect hosts were found. Mermithid nematodes were found to induce a shift from flying to 'drifting' behaviour (floating on top of water) in mayflies (Williams et al., 2001), but this would limit rather than enhance the hosts dispersal ability, as it renders the insect more susceptible to predation by fish. Similarly, mites are known to hinder both survival and flight ability in dragonfly hosts (Forbes et al., 2004), making it unlikely that mite infestation enhances dispersal in *G. calmariensis*.

Finally, it may simply be that mites and nematodes are present at some sites and not present at others, because of biogeographical reasons not considered in this study. It is impossible to say from this study whether these macroparasites have a homogenous distribution and whether the observed effect of site age would persist if that were the case. If they are generalists, then their distribution would be influenced by the abundance of alternative host beetles.

Unlike both macroparasites, gregarine parasites were more abundant at older sites. This provides both anecdotal evidence in support of the enemy release hypothesis, and concrete evidence that accumulation of parasites can occur quickly. Beetle populations at the oldest release sites have been present for only 15 years, a relatively short amount of time. Studies comparing native and introduced species, or comparing invasive species in their introduced and native ranges, often use introduced species which have been established for much longer than this, up to hundreds of years (Agrawal & Kotanen, 2002, Agrawal et al., 2005, Carpenter & Cappuccino, 2005). It may be that this is too

long a time frame, as species will have acquired many natural enemies in their introduced range.

Other examples of natural-enemy acquisition over a similar time frame exist in the literature. The invasive fire ant, *Solenopsis invicta* has decreased in population density and general 'invasiveness' approximately ten years after establishment of a new population in its introduced range. The microsporidian parasite *Thelohania solenopsea* is thought to be responsible for limiting this species (Morrison, 2002). Similarly, predatory mites are thought to be responsible for the ineffectiveness of the biocontrol agent of gorse, *Ulex* sp. in the central United States. The spider mite, *Tetranychus lintearius* was introduced to control this invasive weed in Oregon in 1994, but was largely unsuccessful because it rarely attains high population densities. A survey for natural enemies of *T. lintearius* found that it had acquired both generalist and specialist (to the genus *Tetranychus*) predatory mites. Exclusion of the generalist predator resulted in improved infestation of gorse plants by *T. lintearius* (Pratt et al, 2003).

There was no way to control for population density of *G. calmariensis* when testing the effect of site age. Recently colonized sites have lower population densities, because they are on the outward edge of the 'wave' of the expanding population. For example in the summer of 2004, a single *G. calmariensis* beetle was found in Petrie Island (N. Cappuccino, pers. comm.). The sites in Chelsea and Lac des Feés were selected because they were approximately the same distance from original release sites as Petrie Island and Rockland. At these sites population density was low at time of collection, but feeding damage on plants indicated that it had previously been in the medium range.

Site age was a significant predictive factor for three fungal species: *Aspergillus* sp., *P. farinosa*, and *M. ansiopliae*. Of these, *P. farinosa* conveys the best information, because of its high prevalence, and because relative date was not a significant predictive factor for this species. The same is true of *Beauvaria bassiana*, but no effect of site age was observed for this species. For *Aspergillus* sp., this result should be viewed with caution because of the strong effect of site age which may be an indication of laboratory contamination. The genus *Aspergillus* is widespread and many species are saprophytic, feeding on dead organisms. The pathogenicity of the *Aspergillus* isolates in this study cannot be confirmed in this host, and because no controls were used to confirm that fungal spores seen originated inside the insect cadavers, the *Aspergillus* sp. data cannot be used to make conclusions about the effects of site age or sex on fungal pathogens.

Because fungi are transmitted by spores which may be easily dispersed and remain viable for a long time, there may be some concern that the potential laboratory contamination seen for *Aspergillus* sp. (above) may occur for the other species observed, as fungal prevalence increases with relative date. This is unlikely for several reasons.

The vials in which beetles were collected and held prior to processing were sterilized between uses in an autoclave, so direct transmission from early-season beetles to late-season beetles is not possible by this vector. Each insect was incubated for fungi individually in a Petri dish, which remained closed during watering and visual inspection for fungi. When microscopic inspections of fungi were performed, this took place at a separate workstation from where insects were processed and incubated. The number of species detected also increased with relative date, which could not occur if lab

contamination were the cause. Different species were abundant at the end of the season than at the beginning. Finally, the effect of relative date on fungal prevalence was found to be due to the increase in *Aspergillus* sp. alone, and was not found in the other species considered.

The fungal infection data do not support the predictions of the enemy release hypothesis. For all species where there was a significant effect of site age, more recently colonized sites had higher fungal infection rates. This provides anecdotal support of the biotic resistance hypothesis, but this support is weak.

Beetle age was a significant predictor of fungal infection for a number of fungal species. This is unexpected because entomopathogenic fungi are usually picked up by insect hosts in soil containing spores (as in Bidochka et al., 1998), when the host drops to the ground to pupate. Thus, it might be expected that all age classes would be equally infected, or that older individuals would have a lower prevalence of fungal infection, as infected individuals die off. However, it was found that overwintered (i.e. collected before the first larvae of the season have pupated) adults, the oldest age class of beetle, had the highest prevalence of fungal infection. This might be attributable to the fact that overwintered adults have spent longer in the soil, and were in contact with potentially infected soil during spring and fall, when humidity is higher. Humidity facilitates the infectiveness of entomopathogenic fungi (James et al., 1995).

Although all of the fungal species identified are known to have some entomopathogenic capacity (albeit weak in the case of *Aspergillus*), there is a large amount of genetic variability within fungal species. Without experimental infection to

verify that these strains meet Koch's postulates (), it must be considered that they may possibly have no disease-causing ability in this host.

The results of the fungal infection data indicate that when considering entomopathogenic fungi as natural enemies in tests of the enemy release hypothesis, the age of the host, and the time of the season must be taken into consideration, as these factors contribute significantly to variation in fungal prevalence.

Because *G. calmariensis* can and do disperse on their own, it might be questioned whether some of the collection sites are in fact separate populations. *G. calmariensis* disperses slowly on its own. This is usual for biocontrol agents in their introduced ranges when food is prevalent (McAvoy et al., 1997), as there is simple no pressure to go elsewhere. As such, dispersal has been very slow. Beetles released in North Bay, ON spread a maximum of 26.4 m through continuous habitat over three years (Dech & Nosko, 2002). A study testing dispersal capability under conditions which would maximize distance covered found that 800m approaches the maximum distance beetles can travel to find food (Grevstad & Herzig, 1997). In this case beetles were released in unsuitable habitat (no *L. salicaria*) at varying distances from suitable habitat, and time to arrival and number of beetles arriving successfully was recorded daily. To cover a distance of 800 m took beetles approximately seven days under optimum flying conditions (warm, calm weather). The sexes did not differ in dispersal capability. Beetles preferred areas where conspecifics were already present. If ideal habitat was within 50m, beetles could orient towards it, but beyond this distance dispersal was random (Grevstad & Herzig, 1997). These data would seem to suggest that beetles have limited dispersal ability, especially across patchy habitat that does not contain *L.*

salicaria. Dispersal may be facilitated along waterways, as beetles that fall into moving water may be carried significant distances before being able to extricate themselves. Unrecorded or unofficial releases of *G. calmariensis* by private groups may also have occurred, as biocontrol of *L. salicaria* has received much press attention. Thus, while it is unlikely that sufficient dispersal occurs for nearby sites to be considered one large site, it cannot be ruled out that certain sites may contain individuals descended from other populations as well as from the originally released population.

Although no empirical observations of predation on *G. calmariensis* were undertaken in this study, some information on this topic is available. At the Kemptville site, an adult beetle was observed being captured and eaten by a spider, tentatively identified as the golden garden spider, *Argiope aurantia*. Grevstad & Herzig (1997) report witnessing dragonfly predation of *G. calmariensis* while making dispersal observations. Ladybird beetles (Coleoptera:Coccinellidae) also represent a significant predation threat to larvae and eggs of *Galerucella* spp. beetles (Wiebe & Obrycki, 2002, Sebolt & Landis, 2002, 2004, Nechols et al., 1996). *Coleomegilla maculata, Coccinella septempunctata* and *Harmonia axyridis* are all significant coccinellid predators of *G. calmariensis*. The Coccinellidae are the most studied and probably most significant predators of *G. calmariensis*, but *Pterostichus melanarius* (Coleoptera: Carabidae), *Podisus maculiventris* (Hemiptera: Pentatomidae), and members of the families Lycosidae, Cleridae, Empididae, Chrysopidae, Nabidae, Mimetidae, Salticidae and Theoridiidae have all been reported to feed on eggs and larvae of *G. calmariensis* (Sebolt & Landis, 2004, Wiebe & Obrycki, 2004). While coccinellid hosts raised on *Galerucella* sp. eggs had lower survival rates, longer developmental times, and lower adult body

weights than those fed on native prey species, they were nonetheless able to complete development successfully on this prey, and *Galerucella pusila* eggs, at least, may serve as secondary prey when food is scarce (Wiebe & Obrycki, 2002). Experimental exclusion of predators results in a significant increase in *Galerucella* spp. survival rates, indicating that predation by generalist predators is significant (Nechols et al., 1996, Sebolt & Landis, 2004).

Host population density was not considered in analyses of gregarines, mites or nematodes for statistical reasons, but it was included in the logistic regression model as a predictor of fungal infection status. It was not found to be a significant predictor for any fungal species except *M. anisopliae*, whose results are suspect due to small sample size (Table 5).

In reviews of the work that has been done on density-dependent parasitism, it has been shown that no relation between host population density and parasitism is the norm, although both positive and negative correlations are also found frequently. Approximately half of all studies have found no relationship, and the remainder are divided more or less equally between positive and negative relationships (Stiling, 1987, Walde & Murdoch, 1988). The difference in outcomes among studies that have found effects of host population density on parasitism is largely related to spatial scale; studies looking at a small spatial scale tend to find inverse relationships, and studies considering a large spatial scale tend to find the opposite, presumably due to differences in searching and processing of hosts by parasites at different spatial scales (Walde & Murdoch, 1988).

Hosts may respond to population density by altering their response to parasitism. Sanders et al. (2005), demonstrated that *Drosophila melanogaster* bred under crowded conditions for multiple generations developed higher resistance to parasitism, which would be an adaptive response if parasitism were positively density dependent. Wilson et al. (2002) found a similar effect for resistance to fungal pathogens when comparing solitary and gregarious phases of the desert locust, an effect called density-dependent prophylaxis. Again, this effect is predicated on the higher per capita risk of becoming infected under crowded conditions (Wilson et al., 2002).

A significant female-oriented sex bias was detected for both species of macroparasite found on *G. calmariensis*, but in both cases the prevalence and intensity of infestation was extremely low. This leads to concerns over whether the statistically significant result is truly significant, and whether there is any biological significance. Although removing data from sites where no macroparasites were found is mathematically defensible and common sense, it must be kept in mind when interpreting the results.

If the nematode worms are indeed mermithids, a female-oriented sex bias might be expected, as mermithids are known to castrate their hosts (Williams et al., 2001). However, a castrated male would still display secondary sexual characteristics, such as the tibial spurs used to sex beetles (Cosse & Allard, 2004) in this study, meaning that observed sex bias is not a result of castration. Female-oriented bias would make evolutionary sense in this case, as castration would create a strong selection pressure in favor of males that are well-defended against this parasite. Williams et al. (2001) found

that mermithid infection led to behavioural changes that increased predation risk in mayflies. Although they did not report sex differences in this regard, it is possible that male *G. calmariensis* may suffer a similar effect, resulting in the observed sex bias.

The female-oriented sex bias seen for mite infestations is similar to several other results reported in the literature. Although in one study male dragonflies were more heavily parasitized by mites (Lajeunesse et al., 2004), there are more examples of female insects being more susceptible to parasitism by mites. This is true of damselflies (Robb & Forbes, 2005), midges, and other species (Edwards & Smith, 2003, and references therein).

Some aquatic mites have been shown to prefer female hosts when given a choice, but this is not a consistent or well understood phenomenon (Edwards & Smith, 2003). In damselflies, as in *G. calmariensis*, females are larger than males (Robb & Forbes, 2005), and this may contribute to the difference as females simply present larger targets for mites. However, damselflies are much larger than *G. calmariensis* (e.g., Anholt, 1997), and the therefore can have a larger size difference between the sexes. Therefore, while size difference may be a valid reason for higher parasitism of females in damselflies, it is unlikely to be the cause for *G. calmariensis*. Furthermore, the assertion that larger hosts carry more parasites is not a hard and fast rule. Forbes & Baker (1990) found that for the damselfly *Enallagma ebrium*, host size had no relation to parasite load for males, and an inverse relationship for females, with smaller and lighter females carrying more mites through from larval to adult stages.

Differences in the development and eventual habitat use patterns have also been considered as explanations for sex-biased parasitism in damselflies (Anholt, 1997, Robb

& Forbes, 2005). Female *Lestes disjunctus* spend more time than males in the larval stage most susceptible to infestation by mites, and are also more active and spend more time in areas where they are likely to interact with mites (Anholt, 1997, Robb & Forbes, 2005). While possibilities such as these cannot be refuted by this study as mechanisms of the sex bias in *G. calmariensis*, it is unlikely that they are the cause. No studies have reported a sex difference in developmental time for this beetle. However, it has not been explicitly studied and could exist. There is no indication that the top soil layers where development from larvae to adult occurs are particularly dangerous with regard to mite attachment, but some other aspect of development could conceivably expose females to greater risk of parasitism by mites. Male and female *G. calmariensis* have not been reported to behave differently when feeding, but again, this has not been explicitly studied.

Female *Enallagma boreale* damselflies experience higher intensity of gregarine infection than do males (Hecker, 1999, Hecker et al., 2002). This is consistent with the results of this study. This study did not examine effects of gregarines on the host. Hecker et al. (2002), looked at the putative effects of gregarines on condition of *E. boreale*, and found that, surprisingly, gregarine load was positively associated with condition (mass statistically corrected for length). They speculated that this is likely due to a third factor, namely food consumption. As gregarines are ingested with food, individuals which feed more will be exposed to more parasites. If gregarines are benign or only mildly debilitating, the net effect will be positive as the advantages of increased resource acquisition outweigh the costs of parasitism.

As female *G. calmariensis* are larger than males, they likely have higher gregarine loads for this very reason, although experimental exposures would be needed to confirm that this is the case. Larger size may also indicate a larger digestive tract capable of accommodating more parasites, although this alone could not account for the large difference seen. The cost of gregarine parasitism on *G. calmariensis* has not been evaluated. The majority of dissected beetles had both gregarines and food in the digestive tract (pers. obs), though in a few individuals with higher (>500) gregarine counts, the digestive tract contained only gregarines and was often distended because of this, suggesting that the blockage mentioned by Siva-Jothy & Plaistow (1999) may occur in some cases. Further studies would be needed to evaluate what, if any, costs gregarines incur in this host.

It is unusual that the gregarines infecting *G. calmariensis* were found in the foregut, as in most cases they are reported in the midgut. If the gregarines are a species of parasite that *G. calmariensis* has acquired since introduction in North America, the unusual placement of the parasites could be a sign of incomplete adaptation. In this case one would expect that over time the parasites would become localized in the midgut as in other species (e.g.. Hecker et al., 2002). Localization in the foregut could also be simply an idiosyncrasy of this host-parasite system. The parasites are able to complete their lifecycle within the host, evidenced by the presence of oocysts, which would be somewhat unusual if it were an example of new and poorly adapted relationship.

When viewing all fungal data together, it appears that males suffered from a higher rate of fungal infection than females ($p=0.04$). When viewed on a species by

species basis, however, this bias disappears, indicating a weak effect. A lack of sex bias in fungal infection is consistent with the findings of Sherridan et al. (2000), who included fungal species in their review of the literature on sex-bias in parasitism, and found no overall effect of sex. However, the weak effect that is seen when all fungal species are grouped together suggests that there may be a very weak effect that can only be detected with a large sample size.

A small percentage (5%) of the insects sampled yielded two species of fungi when assayed. This is in comparison to the results of Bidochka et al. (1998), wherein 1.9% to 6.2% of their soil samples (depending on incubation temperature) yielded two fungal species, but multiple infections were never observed in waxworm larvae incubated with those soil samples. Why there is a difference, and what the significance of multiple infections is, is unclear.

Conclusions

This study provides anecdotal evidence that supports the ERH where gregarine parasites are concerned. The remainder of the data do not support the predictions of the ERH, although there is some evidence in favor of the biotic resistance hypothesis. Although this study does not have the breadth to provided a proper experimental test of either hypothesis, it still has the ability to inform issues that may influence the outcome of such studies and may otherwise be overlooked.

This study indicates that when testing the assumptions of the ERH, factors such as host age and relative date must be taken into consideration, and that care should be taken to balance the sex ratios when comparing populations. It also indicates that the 50 – 300+ year scale often used when comparing enemies of invasive species to either their native counterparts or populations in their native range may be too large, as beetles in this study had accumulated significant parasites loads in fifteen years or less. Location within the introduced range must also be considered, as significant site-age related variation was found for this species.

In general, there is evidence for a female-oriented sex bias in parasitism of *G. calmariensis*. This is most true of eugregarine gut parasites, which may be present in high enough numbers to cause mortality in this host. Although highly significant p values were obtained for comparisons of nematode worms and *Leptus* sp. mites, the small number of sites where these parasites were found, and the low prevalence at these sites, make these findings less reliable. There is no evidence for sex bias in prevalence of entomopathogenic fungi for any of the fungal species detected in this study.

References

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

Agrawal, A.A., Kotanen, P.M., Mitchell, C.E. (2005). *Enemy release? An experiment with congeneric plant pairs and diverse above- and beloground enemies.* Ecology, vol. 86(11):2279-2989.

Anholt, B.R. (1997). *Sexual size dimorphism and sex-specific survival in adults of the damselfly* Lestes disjunctus. Ecological Entomology, vol. 22: 127-132.

Bidochka, M.J., Kasperski, J.E. & Wild, G.A.M. (1998). *Occurrence of the entomopathogenic fungi* Metarhizium anisopliae *and* Beauveria bassiana *in soils from temperate and near-northern habitats.* Canadian Journal of Botany, vol. 76:1198-1204.

Blaney, C.S. & Kotanen, P.M. (2001). *Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs.* Journal of Applied Ecology, vo. 38: 1104-1113.

Blossey, B. & Notzold, R. (1995). *Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis.* The Journal of Ecology, vol. 83(5), 887-889.

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

Bouwma, A.M., Howard, K.J., Jeanne, R.L. (2005). *Parasitism in a social wasp: effct of gregarines on foraging behavior, colony productivity, and adult mortality.* Behavioural Ecology and Sociobiology, vol. 59: 222-233.

Carpenter, D. & Cappuccino, N. (2005). *Herbivory, time since introduction and in the invasiveness of exotic plants.* Journal of Ecology, vol. 93: 315-321.

Colautti, R.I., Ricciardi, A., Grigorovich, I.A., & MacIsaac, H.J. (2004). *Is invasion success explained by the enemy release hypothesis?* Ecology Letters, vol. 7: 721-733.

Corrigan, J. (2004). *"Ten Years After" – a Summary of Results for the 2004 Tour of Release Sites from the 1992-1997* Galerucella spp. *Biological Control Programs in Ontario.* Prepared by:Bio-Logical Alternatives, Harriston, ON, for Ontario Ministry of Natural Resources.

Cosse, A.A. (2004). *The presence of tibial spurs as a male sexual characteristic for* Galerucella calmariensis, *(Coleoptera:Chrysomelidae).* Journal of Entomological Science, vol. 39(2): 281-283.

Creighton, C.S. & F. Fassuliotis. 1983. *Infectivity and suppression of the banded cucumber beetle (Coleoptera: Chrysomelidae) by the mermithid nematode* Filipjevimermis leipsandra *(Mermithida: Mermithidae).* Journal of Economic Entomology, vol.76:615-618.

Davis, M.A., & Thompson, K. (2001). *Invasion terminology: should ecologists define their terms differently than others? No, not if we want to be of any help!.* Bulletin of the Ecological Society of America, vol. 82:206.

Dech, J.P., & Nosko, P. (2002). *Population establishment, dispersal and impact of* Galerucella pusilla *and* G. calmariensis, *introduced to control purple loosestrife in central Ontario.* Biological Control, vol. 23: 228-236.

De Moraes, A.M.L., Da Costa, G.L., De Camargo Barcellos, M.Z., De Oliviera, R.L., De Oliviera, P.C. (2001). *The entomopathogenic potential of* Aspergillus *spp. in mosquitoes vectores of tropical diseases.* Journal of Basic Microbiology, vol. 41(1): 45-49.

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

DeWalt, S.J., Denslow, J.S. & Ickes, K. (2002). *Natural enemy release facilititates habitat expansion of the invasive tropical shrub* Clidemia hirta. Ecology, vol. 85, 471-483.

Dietz, H., Wirth, L.R., Buschmann, H. (2004). *Variation in herbivore damage to invasive and native woody plant species in open forest vegetation on Mahé, Seychelles.* Biological Invasions, vol. 6: 511-521.

Drummond, J. & Pinnock, D.F. (1990). *Aflatoxin production by entomopathogenic isolates of* Aspergillus parasiticus *and* Aspergillus flavus. Journal of Invertebrate Pathology, vol. 55(3): 332-336.

Edwards, D.D. & Smith, H.G. (2003). *Host sex preferences and tranmission success by the water mite* Unionicola foili *(Acari: Unionicolidae) parasitic on the midge* Chironomus tentans *(Diptera:Chrionomidae).* Journal of Parasitology, vol. 89(4): 681-685.

Ehler, L.E. (1998). *Invasion biology and biological control.* Biological Control, vol 13: 127-133.

Er, M.K. & Gökçe,A. (2005).*Effect of* Diplocystis tipulae *Sherlock (Eugregarinida: Apicomplexa), a coelomic gregarine pathogen of tipulids, on the larval size of* Tipula paludosa *Meigen (Tipulidae: Diptera).* Journal of Invertebrate Pathology, vol. 89: 112-115.

Forbes, M.R. & Baker, R.L. (1990). *Susceptibility to parasitism: experiments with the damselfly* Enallagma ebrium *(Odonata:Coenargrionidae)* and larval water mites, Arrenurus *spp. (Acari:Arrenuridae).* Oikos, vol 58: 61-66.

Fobres, M.R. & Baker, R.L. (1991). *Condition and fecundity of the damselfly,* Enallagma ebrium *(Hagen): the importance of ectoparasites.* Oecologia, vol. 86(335-341).

Forbes, M.R., Muma, K.E. & Smith, B.P. (2004). *Recapture of male and female dragonflies in relation to parasitism by mites, time of season, wing length and wing cell symmetry.* Experimental and Applied Acarlology, vol. 34: 79-93.

Grevstad, F.S. & Herzig, A.L. (1997). *Quantifying the effects of distance and conspecifics on colonization: experiments and models using the loosestrife leaf beetle,* Galerucella calmariensis. Oecologia, vol. 110, 60-68.

Hairston, N.G., Smith, F.E., Slobodkin, LB. (1960). *Community structure, population control, and competition.* The American Naturalist, vol. XCIV (879): 421-425.

Hastings, A., Harrison, A., McCann, K. (1997). *Unexpected spatial patterns in an insect outbreak match a predator diffusion model.* Proceedings of the Royal Society of London: Biological Sciences, vol. 264 (1389), 1837-1840.

Hecker, K.R (1999 Fall). *Testing for sex biases and morph biases in parasitism of zygopterans (Odonata) by gregarines (Eugregarinidae).* Master's Thesis, Ottawa-Carleton Institute of Biology.

Hecker, K.R., Forbes, M.R., Léonard, N.J. (2002). *Parasitism of damselflies (*Enallagma boreale*) by gregarines: sex biases and relations to adults survivorship.* Canadian Journal of Zoology, vol. 80: 162-168.

Henne, D.C., Lindgren, C.J., Gabor, T.S., Murkin, H.R. & Roughley, R.E. (2005). *An integrated management strategy for the control of purple loosestrife* Lythrum salicaria L. *(Lythraceae) in the Netley-Libau March, southern Manitoba.* Biological Control, vol. 32: 319-325.

Hight, S.D., Blossey, B., Laing, J. & Declerck-Floate, R. (1995). *Establishment of insect biologican control agents from Europe against* Lythrum salicaria *in North America.* Biological Control, vol. 24(4): 967 – 977.

James, R.R., Shaffer, B.T., Croft, B., & Lighthart, B. (1995). *Field evaluation of* Beauvaria bassiana*: its persistence and effects on the pea aphid and a non-target coccinellid in alfalfa.* Biocontrol Science and Technology, vol. 5(4): 425 – 437.

Joshi, J., & Vrieling, K. (2005). *The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores.* Ecology Letters, vol. 8(7): 704-714.

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

Kaufman, L.N. & Landis, D.A. (2000). *Host specificity testing of* Galerucella calmariensis *L. (Coleoptera: Chrysomelidae) on wild and ornamental plant species.* Biological Control, vol. 18: 157-164.

Keane, R.M., & Crawley, M.J. (2002). *Exotic plant invasions and the enemy release hypothesis.* TRENDS in Ecology & Evolution, vol. 17(4): 164 – 170.

Kendrick, B. (1992). *The fifth kingdom, 2nd Ed.* Focus Information Group, Newburyport, MA, USA. ISBN 0-941051-28-5.

Kolar, C.S., & Lodge, D.M. (2001). *Progress in invasion biology: predicting invaders.* TRENDS in Ecology & Evolution, vol. 16(4): 199 – 204.

Lajeunesse, M.J., Forbes, M.R., & Smith, B.P. (2004). *Species and sex biases in ectoparasitism of dragonflies by mites.* Oikos, vol. 106:501-508.

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

Lindgren, C.J. (2003). *Using 1-min scans and stem height data in a post-release monitoring strategy for* Galerucella calmariensis *(L.)(Coleoptera: Chrysomelidae) on purple loosestrife,* Lythrum salicaria *L. (Lythraceae), in Manitoba.* Biological Control, vol. 27: 201-209.

Malecki, R.A., Blossey, B.B., Hight, S.D., Schroeder, D., Kok, L.T., Coulson, J.R. 1993). *Biological control of purple loosestrife.* BioScience, vol. 43(10): 680-86.

Manguin, S., White, R., Blossey, B. & Hight, S.D. (1993). *Genetics, taxonomy, and ecology of certain species of Galerugella (Coleoptera: Chrysomelidae).* Annals of the Entomological Society of America, vol. 86(4): 397-410.

Maron, J., L. & Vilà, M. (2001). *When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses.* Oikos, vo. 95: 361-373.

Matsumoto, T., Itioka, T., Nishida, T & Inoue, T. (2004). *A test of temporal and spatial density dependence in the parasitism rates of introduced parasitoids on host, the arrowhead scale (*Unaspis yanonensis*) in stable host-parasitoids system.* Journal of Applied Entomology, vol. 128(4):267-272.

McAvoy, T.J. & Kok, L.T. (2004). *Temperature dependent development and survivial of two sympatric species,* Galerucella calmariensis *and* G. pusilla *on purple loosestrife.* BioControl, vol. 49: 467-480.

McAvoy, T.J., Kok, L.T., & Mays, W.T. (1997). *Phenology of an established population of* Galerucella calmariensis *(L.) and* G. pusilla *(Duft.) (Coleoptera: Chrysomelidae) on purple loosestrife,* Lythrum salicaria *L. (Lythraceae) in Southwest Virginia.* Biological Control, vol. 9: 106-111.

McCurdy, D.G., Shutler, D., Mullie, A., & Forbes, M.R. (1998). *Sex-biased parasitism of avian hosts: relations to blood parasites taxon and mating systems.* Oikos, 82: 303-312.

Mitchell, C.E. & Power, A. G. (2003). *Release of invasive plants from fungal and viral pathogens.* Nature, vol. 421 (6923): 625-627.

Moore, S. L & Wilson,K. (2002). *Parasites as a viability cost of sexual selection in natural populations of mammals.* Science, vol. 297: 2015-2018.

Morrison, L.W. (2002). *Long-term impacts on and arthropod-community invasion by the imported fire ant,* Solenopsis invicta. Ecology, vol. 83 (8), 2337-23345.

Nechols, J.R., Obrycki, J.J., Tauber, C.A. & Tauber, M.J. (1996). *Potential impact of native natural enemies on* Galerucella *spp. (Coleoptera:Chrysomelidae) imported for biological control of purple loosestrife: a field evaluation.* Biological Control, vol. 7: 60-66.

Pell, J.K. & Vandenberg, J.D. (2002). *Interations amond the aphid* Diuraphis noxia, *the entomopathogenic fungus* Paeciliomyces fumosoroseus *and the coccinellid* Hippodamia convergens. Vol. 12(2): 217 – 224.

Poulin, R. (1996). *Sexual inequalities in helminth infection: a cost of being male?* American Naturalist, vol. 147(2): 287-295.

Pratt, P.D., Coombs, E.M., Croft, B.A. (2003). *Predation by phytoseiid mites on* Tetranychus lintearius *(Acari: Tetranychidae), an established weed biological control agent of gorse* (Ulex europaeus). Biological Control, vol. 26, 40-47.

Puterka, G.J. (1999). *Fungal pathogens for arthropod pest control in orchard systems: mycoinsecticidal approach for pear psylla control.* Biocontrol – Journal for the International Organisation for Biological Control, vol. 44(2): 183 – 210.

Richardson, D.M., Pyšek, P., Rejmánek, M., Barbour, M.G., Panetta, F.D., West, C.J. (2000). *Naturalization and invasion of alien plants: concepts and definitions.* Diveristy and Distributions, vol. 6: 93 –107.

Robb, T. & Forbes, M.R. (2005). *Sex biases in parasitism of invertebrate hosts: a case tudy of mite parasitism of the damselfly* Lestes disjunctus. EcoScience, vol. 13(1).

Rolff J. (2000). *Water mite parasitism in damselflies during emergence: two hosts, one pattern*. Ecography, vol. 23: 273-282.

Rolff, J. (2001). *Effects of age and gender on immune function of dragonflies (Odonata, Lestidae) from a wild population.* Canadian Journal of Zoology, vol. 79: 2176-2180.

Rohlfs, M & Hoffmeister, T.S. (2004). *Spatial aggregation across ephemeral resource patches in insect communities: an adaptive response to natural enemies?* Oecologia, vol. 140(4):654-661.

Sanders, A.E., Scarborough, C., Layen, S.J., Kraaijeveld, A.R. & Godfary, H.C.J. (2005). *Evolutionary change in parasitoid resistance under croded conditions in* Drosophila melanogaster. Evolution, vol 59(6): 1292-1299.

Schalk, G. & Forbes, M.R. (1997). *Male biases in parasitism of mammals: effects of study type, host age, and parasite taxon.* Oikos, 78: 67-74.

Sebolt, D.C., & Landis, D.A. (2002). *Neonate* Galerucella calmariensis *(Coleoptera: Chrysomelidae) behavior on purple loosestrife* (Lythrum salicaria) *contributes to reduced predataion*. Environmental Entomology, vo. 31 (5), 880-886.

Sebolt, D.C. & Landis, D.A. (2004). *Arthropod predators of* Galerucella calmariensis *L. (Coleoptera: Chrysomelidae)L: an assesment of biotic interference.* Environmental Entomology, vol. 33(2):356-361.

Sheridan, L.A.D., Poulin, R., Ward, D.F., & Zuk, M. (2000). *Sex differences in parasitism among arthropod hosts: is there a male bias?* Oikos, vol. 88: 327-334.

Siva-Jothy, M.T. & Plaistow, S.J. (1999). *A fitness cost of eugegarine parasitism in a damselfly.* Ecological Entomology, vol. 24: 465-470.

Smith, B.P. (1988). *Host-parasite interaction and impact of larval water mites on insects.* Annual Review of Entomology, vol. 33, 487-507.

Stewart, T. (1998). *Detailed taxonomy of parasitic helminthes.* www.path.cam.ac.uk/~schisto/Taxonomy/Old.Taxonomy.html. Cambridge University.

Stiling, P.D. (1987). *The frequency of density dependence in insect host-parasitoid systems.* Ecology, vol. 68(4): 844-856.

Strasser, H., Vey, A. & Butt, T.M. (2000). *Are there any risks in using entomopathogenic fungi for pest control, with particular references to the bioactive metabolits of* Metarhizium, Tolypocladium, *and* Beauvaria *species?* Biocontrol Science and Technology, vol. 10 (6): 717 - 736

Thompson, D.Q., Stuckey, R.L., and Thompson, E.B., 1987. *"Spread, Impact and Control of Purple Loosestrife (*Lythrum salicaria*) in North American Wetlands."* Fish and Wildlife Res. 2, U.S. Dept. Interior, Washington, DC.

Todorova, S.I., Côté, J-C, Martel, P., Coderre, D. (1994). *Heterogeneity of two* Beauvaria bassiana *strains revealed by biochemical tests, protein profiles, and bio-assays on* Leptinotaros decemlineata *(Col:Chrysomelidae) and* Coleomegilla maculata *Lengi (Col: Coccinellidae) larvae.* Entomophaga, vol 39(2): 159-169.

Todorova, S.I., Côté, J-C, Codere, D. (1996). *Evaluation of two* Beauvaria bassiana *(Balsamo) Vuillemin strains on the development of* Coleomegilla maculata *Lengi*

Timberlake (Col: Coccinellidae). Journal of Applied Entomology, vol. 120(3): 159 – 163.

Torchin, M.E., Lafferty, K.D., Kuris, A.M. (2001). *Release from parasites as natural enemies: increased performance of a globally introduced marine crab.* Biological Invasions, vol. 3: 333 – 345.

Torchin, M.E., Lafferty, K.B., Dobson, A.P., McKenzie, V.J., Kuris, A.M. (2003). *Introduced species and their missing parasites.* Nature, vol. 421, 628-630.

Vilà, M., Maron, J.L. & Marco, L. (2005). *Evidence for the enemy release hypothesis in* Hypericum perforatum. Oecologia, vol. 142: 474-479.

Walde, S.J. & Murdoch, W.W. (1988). *Spatial density dependence in parasitoids.* Annual Review of Entomology, vol. 33, 441-466.

Wedekind, C., & Jakobsen, P.J. (1998). *Male-biased suceptibility to helminth infection: an experimental test with a copepod.* Oikos, vol. 81: 458-462.

Wiebe, A.P & Obrycki, J.J. (2002). *Prey suitability of* Galerucella pusilla *eggs for two generalist predators,* Coleomegilla maculata *and* Chrysoperla carnea. Biological Control, vol. 23: 143-148.

Wiebe, A.P. & Obrycki, J.J. (2004). *Quantitative assessment of predation of eggs and larvae of* Galerucella pusilla *in Iowa.* Biological Control, vo. 31: 16-28.

Williams, J.K., Townsend, L.R., & Poulin, R. (2001). *Mermithid nematode infections and drift in the mayfly* Deleatidium *spp. (Ephemeroptera).* Journal of Parasitology, vol. 87(5): 1225-1227.

Wilson, K., Thomas, M.B., Blanford, S., Doggett, M., Simpson, S.J., Moore, S.L. (2002). *Coping with crowds: denisty-dependent disease resistance in desert locusts.*

Zuk, M. &McKean, K.A. (1996). *Sex differences in parasite infections: patterns and processes.* International Journal for Parasitology, vol. 26(10): 1009-1024.

Zuk, M & Stoehr, A.M. (2002). *Immune defense and host life history.* The American Naturalist, vol. 160, supplement: S9-S22.

Appendix 1: Glossary of Terms

Biological Control (Biocontrol): The practice of releasing natural enemies in order to control pest species, native or otherwise. These natural enemies are referred to as biological control agents or biocontrol agents.

Biotic Resistance Hypothesis: An explanation for why most introduced species do not become invasive. The BRH states that competition with better adapted native species and interference by native natural enemies limits the expansion of most introduced species.

Ectoparasite/Endoparasite: An ectoparasite is one which feeds and lives inside the host. An endoparasite feeds and lives outside of the host, but attached to it in some way.

Enemy Release: When a species is freed from constraints imposed by natural enemies. This can be natural, as when a species colonizes a new area without its natural enemies, or artificial, as when a species is released after quarantine to remove natural enemies.

Enemy Release Hypothesis: An explanation for why some introduced species become invasive. The ERH states that invasive species experience a release from natural enemies upon colonizing new areas, thereby allowing them to devote resources to growth and reproduction instead of defense, and affording them an advantage over native species.

Exotic Species: Non-native species which may become invasive but are not necessarily so.

Gregarine/Eugregarine: A member of the order Eugregarinidae, which consists of gut parasites of arthropods.

Introduced Range: The non-native range of a species. If the species is considered invasive, the introduced range is typically where it is a pest.

Introduced Species: A species that is not native to a given area and has been introduced, accidentally or by design from a geographically removed location (such as across an ocean).

Invasive Species: A species which has not only become established in a novel range, but is spreading and dominating its new habitat, causing a significant alteration of the ecosystem it inhabits.

Invasiveness: A somewhat subjective term denoting the ability of an introduced species to become invasive, and the degree to which an invasive species exemplifies that definition.

Macroparasite/Microparasite: A non-evolutionary grouping of parasites. Macroparasites are large and can be seen with the naked eye. Microparasites are small and must be observed under magnification. Pathogens such as fungi may be considered microparasites.

Mermithid: A member of the order Mermithida, consisting of nematode worms which parasitize insects.

Native Range: The original range where founding populations of introduced species originate from. Invasive species may or may not be pests in their native range.

Natural Enemies: The suite of pathogens, parasites, parasitoids, predators, etc. which affect a species, either in its native range or introduced range.

Non-Native Species: Used here as a synonym for Introduced Species. Species which is presently found in a place geographically removed from its native range.

Sex Biased Parasitism: The condition where one sex of a host species experiences a greater prevalence or intensity of parasitism by a specific parasite or suite of similar parasites.

Appendix 2: Natural History of Parasite Species

Gregarines (Apicomplexa:Eugregarinidae)

Gregarines are a diverse and poorly classified order of parasites. They are widespread, with a diversity of species infecting a diversity of invertebrate hosts. In a few well-studied host-parasite systems, the gregarine species are identifiable and well defined (Er & Gökçe, 2005), but often in the literature gregarines go unidentified (e.g., Hecker et al., 2002). Gregarines are protozoan, extracellular gut parasites, and they are exclusive to invertebrates (Bouwma et al., 2005, Siva-Jothy & Plaistow, 1999). They are often the predominant parasite of a given species (Siva-Jothy & Plaistow, 1999) and can achieve high densities. Virulence ranges from benign commensalism to debilitating parasitism. Fatalities are rare (Bouwma et al., 2005) but may occur in some species at high densities (Er & Gökçe, 2005). This is likely due to blockage or rupturing of the digestive tract, which hinders feeding (Siva-Jothy & Plaistow, 1999).

The life cycle of a gregarine begins when the host ingests oocysts on contaminated food. The oocysts 'hatch' into numerous sporozoites, which then attach to the gut (usually mid-gut) epithelium and begin to feed and grow. They mature into trophozoites, which may then fuse into gametocysts, undergo sexual reproduction, and produce more oocysts (Siva-Jothy & Plaistow, 1999). These are then usually expelled, but they may remain in the host and repeat the cycle. Gregarines can also undergo asexual reproduction by simply breaking off segments of the body in a process called fission (Hecker, 2002).

Nematodes

The phylum Nematoda is undergoing revision and sometimes poorly defined. Mermithids (Mermithida:Mermithidae) are sexually reproducing parasites of insects (Stewart, 1998). Although the nematode in this study remains unidentified, another species, *Filipjevmeris leipsandra* is known to parasitize the chrysomelid *Diabrotica balteata*, the banded cucumber beetle, and is used as a biocontrol agent for this invasive pest (Creighton & Fassuliotis, 1983).

Entomopathogenic Fungi

Entomopathogenic fungi are a group of species that have received much attention recently for their potential as biocontrol agents. They have advantages over pesticides in that they are less toxic, reproduce quickly and abundantly, and can remain dormant but still potentially infectious even when hosts are rare, helping to prevent the dramatic resurgence of a pest that is often seen after application of chemical insecticides (Kendrick, 1992). They also have disadvantages, in that they are often slow to act or produce only moderate reductions in pest levels compared to chemical pesticides, and may be pathogenic to non-target, beneficial organisms as well (Kendrick, 1992). Numerous strains of various fungal species have been commercially developed as mycoinsecticides around the world (Strasser et al., 2000, Puterka, 1999) and more are continually being developed and tested for host specificity, toxicity and effectiveness. Entomopathogenic fungi are generally distributed widely on a global scale, and have broad host ranges (James et al., 1995).

The genus *Aspergillus* (Eurotiales:Trichocomamaeae) is a blue mold related to *Penicillium* spp. (Kendrick, 1992), with many species. No *Aspergillus* spp. are exclusively entomopathogenic, but a large number of them can be pathogenic under the right conditions. The genus has received much attention in the literature because of its usefulness in biotechnology and medical applications, as well as its potential as a biocontrol agent for insects, specifically mosquitoes, which may be infected with any one of a large number of species (De Moraes et al., 2001, Drummond & Pinnock, 1990). *Aspergillus* spp. produce many secondary metabolites that may be toxic to insects (De Moraes et al., 2001). Because the *Aspergillus* sp. isolated in this study cannot be identified to the species level, and because the genus is so broad, it cannot be used to make statements about entomopathogenic fungi.

Beauvaria bassiana (Clavicipitales:Claviceptaceae) is one of the most widely studied entomopathogenic fungi, and the most broadly used in commercial mycoinsecticides (Puterka, 1999). *B. bassiana* infects all major orders of insects, and is one of the most widespread of the entomopathogenic fungi, being found all over the world, including an island which is part of Antarctica (James et al., 1995, Todorova et al., 1994). Strains of *B. bassiana* can vary significantly in pathogenicity (Todorovova et al., 1996), and this variation is related to which host it is infecting. A given insect may be highly vulnerable to one strain and highly resistant to another, while another closely related insect species may experience the reverse (Todorova et al., 1994). *B. bassiana* is one of the most common entomopathogenic fungal species recovered from soil samples in Ontario (Bidochka et al., 1998).

Metarhizium anisopliae (Eurotiales:Hypocreales) is another of the most common entomopathogenic fungi, and also one of the most common in soils in Ontario (Bidochka et al., 1998). It is more likely to be recovered from soils in disturbed habitats than natural ones (unlike *B. bassiana* which is the reverse) and is recovered more at higher incubation temperatures than *B. bassiana*, which is moderately psychrophillic (prefers low temperatures) (Bidochka et al., 1998). *M. anisopliae* has a wide distribution and host range (Bidochka et al., 1998) and has been developed commercially for biocontrol of weevils, scarabs, spittle bugs, cockroaches and termites (Strasser et al., 2000). Sometimes used as a mycoinsecticide along with *M. anisopliae* is the congeneric species *M. flavoviridae* (Puterka, 1999), and it is possible that this is the identity of the unknown species in this study, as it resembles *M. anisopliae.*

The genus *Paeciliomyces* (Eurotiales:Trichocomamaeae) has two entomopathogenic members which are common in Ontario, *P. farinosa* and *P. fumosoroseus*. Together with *B. bassiana* and *M. anisopliae*, these species made up 99% of entomopathogenic fungal isolates obtained from soil samples in the province (Bidochka et al., 1998). This is consistent with the results of this study, except that *P. fumosoroseus* was recovered less often than *Aspergillus* sp. in this case. *P. fumosoroseus* has been developed commercially to control orchard pests (Puterka, 1999) and pests in greenhouses (Pell & Vandenberg, 2002).