Competitive interactions among two specialist predators and a generalist predator of hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae)

Robbie W. Flowers

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Scott M. Salom, Co-Chair
Loke T. Kok, Co-Chair
Douglas G. Pfeiffer
Donald E. Mullins
Michael E. Montgomery

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Abstract

Competitive interactions among two specialist predators, *Laricobius nigrinus* Fender and *Sasajiscymnus (Pseudoscymnus) tsugae* Sasaji and McClure, and a generalist predator, *Harmonia axyridis* Pallas, of hemlock woolly adelgid were evaluated in the laboratory and field. The two specialist predators are part of a biological control program for *Adelges tsugae* Annand, and the potential for competition among these species and previously established generalist predators is unknown. In addition, detailed studies of predator behavior and activity patterns were completed using video methods.

In laboratory assays, predator feeding on conspecific and heterospecific predator eggs was tested at three *A. tsugae* densities. Eggs of *L. nigrinus* and *S. tsugae* were preyed upon by all species, and predation decreased with increased *A. tsugae* density. Eggs of *H. axyridis* were consumed almost exclusively by conspecifics, at high rates, for all *A. tsugae* densities. Predator survival, net egg production and feeding on *A. tsugae* were evaluated for single- and multiple-predator conspecific and heterospecific groupings at simulated early and late spring conditions. Survival was not significantly affected by the presence of additional predators or environmental conditions. Net egg production was significantly reduced in conspecific groupings, but was unaffected by heterospecifics. Conspecific groupings did not significantly affect feeding on *A. tsugae* by *L. nigrinus* or *S. tsugae*, but *H. axyridis* showed significant reductions. In contrast, feeding on *A. tsugae* by all species was not significantly affected by heterospecific groupings. Each species showed increased net egg production and feeding on *A. tsugae* at higher temperature assays, but this was proportionately greater for *S. tsugae* and *H. axyridis*. The only significant negative competitive interactions detected were among conspecifics, while heterospecific combinations showed non-interference.

In field assays, adult predators were evaluated using branch cages during spring and summer at two field sites infested with *A. tsugae*. Using females only in 2003 and sexual pairs in 2004, we examined predator survival and net reproduction as well as predator feeding and impact on *A. tsugae* in single- and multiple-predator conspecific and heterospecific groupings. Predator survival was high overall and not affected by the presence of additional predators during either evaluation. Net reproduction per predator was negatively affected by conspecifics, but unaffected by heterospecifics. The progeny of *L. nigrinus* were more developmentally advanced than the other species in spring, indicating earlier reproductive activity. Net reproduction by predators was similar in both years of the study, suggesting that environmental conditions are the limiting factors for reproduction in these species. Total feeding was greater for all species when placed in predator groupings, suggesting that interactions may not significantly interfere with feeding activity. Feeding by *S. tsugae* and *H. axyridis* was greater during the summer evaluation, indicating increased activity under these conditions. Predator treatments reduced the number of developing or aestivating *A. tsugae* nymphs relative to controls during each evaluation. In spring, *L. nigrinus* had the greatest impact on *A. tsugae*, and *H. axyridis* had the greatest impact during summer. Predator impact was greater during both evaluations in the second year of the study, possibly due to increased activity by predators in the more favorable environmental conditions. Overall, it appears that it is the production of predator progeny and their subsequent activity that has the greatest impact on future *A. tsugae* generations.

In video studies, adult predator behavior and daily activity patterns were documented using single- and multiple-predator groupings under simulated spring and summer conditions. Digital video recordings were captured at regular intervals over 24 h and examined to describe predator behavior as well as daily temporal and spatial activity patterns. Predator behavior varied qualitatively and quantitatively by species, and did not appear to be coordinated to any particular time or location. All species exhibited continuous activity patterns that were punctuated by longer periods of rest. Each species displayed extensive and intensive searching patterns, with intensive searching more varied by species. The specialist predators, *L. nigrinus* and *S. tsugae*, were more selective of feeding and oviposition sites, and rested at more concealed locations than *H. axyridis*. Under spring conditions, *L. nigrinus* was more active and had a more even daily behavior distribution than *S. tsugae* or *H. axyridis*, which were skewed towards resting. In summer conditions, a more even daily behavior pattern was observed for *S. tsugae* and *H. axyridis* in response to increased temperatures. Multiple-predator assays indicated that conspecifics significantly altered the time allocated to behaviors for *L. nigrinus* and *H. axyridis*, leading to increased searching behaviors and decreased feeding, oviposition and resting. In contrast, *S. tsugae* conspecifics and heterospecific combinations did not significantly affect behavior patterns. All species maintained a high degree of spatial separation relative to assay size. Distances were greatest between conspecifics of *H. axyridis* and *L. nigrinus*, followed by heterospecific combinations and *S. tsugae* conspecifics. Spatial separation among these species may be regulated by avoidance behaviors in response to chemical or tactile (direct contact) cues.

Taken together, these studies suggest that these three species will be compatible within a biological control program for *A. tsugae*. Interspecific interference among these predators did not occur to a significant degree in Petri dish assays in the laboratory or branch enclosures in the field. In addition, video studies indicate that predator temporal and spatial patterns were not coordinated, such that significant interference may not occur under more natural conditions. In contrast, intraspecific interference negatively affects these species and may have important implications for release strategies. Overall, the use of multiple predator species combinations is recommended over single species when implementing biological control for *A. tsugae*. Low-density releases are also recommended to reduce the potential negative effects of intraspecific competition.

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Preface

There is some repetition in the following chapters as each was prepared as a separate manuscript for publication in scientific journals.

Chapter 1

Introduction and Literature Review

Adelges tsugae in the eastern United States

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a major pest of eastern hemlock, *Tsuga canadensis* L. Carriere, and Carolina hemlock, *T. caroliniana* Engelmann, in the eastern United States. Heavily infested trees exhibit poor crown health and reduced shoot growth that, in combination with other environmental stresses, can result in rapid tree decline and death (McClure 1987, Mayer et al. 2002, Orwig 2002). The geographic range of eastern hemlock extends north to Nova Scotia, west to Minnesota and south to northern Alabama (Baumgras et al. 1999). *Adelges tsugae* in the eastern United States is believed to originate from Japan, where it is an innocuous inhabitant of *Tsuga spp.* (Havill et al. 2006), and where populations are likely regulated by host resistance and natural enemies (McClure et al. 2001).

This species was first observed in the 1950s in a landscape setting in northern Virginia and has since spread from Georgia to southern Maine, causing widespread mortality of hemlock species (Cheah et al. 2004). It is rapidly dispersed through wind as well as by bird, small mammal and human movements (McClure 1990). It has an estimated spread of 25-30 km per year (McClure et al. 2001). In eastern North America, *A. tsugae* populations rapidly colonize and quickly compromise tree health due to the absence of effective natural enemies (Cheah and McClure 1998, Wallace and Hain 2000), their ability to survive at low temperatures and the high susceptibility of eastern hemlock species (Parker et al. 1999).

These characteristics may allow *A. tsugae* to spread throughout the native range of eastern hemlock (McClure et al. 2001); however, it appears that cold temperature may limit its northern progression (Skinner et al. 2003).

Hemlocks in eastern North America are a valuable resource both environmentally and commercially. Perhaps their greatest value lies in the importance of the ecological niche they occupy as a component of eastern forests. Hemlock occupies about 800,000 ha of forest in pure or mixed *T. canadensis* stands (Orwig and Foster 1998). As a climax species, it is extremely shade-tolerant and creates a unique microclimate that provides unique habitats for terrestrial and aquatic plant and animal species (Evans et al. 1996, Ward et al. 2004). In addition, hemlock helps to maintain aquatic ecosystems and watersheds by regulating stream flows and moderating water temperatures (Evans et al. 2002). The large-scale mortality of hemlock will undoubtedly change forest structure, composition and resource availability within its geographic range (Foster 1999). Hemlock is also valued economically for commercial uses and its aesthetic appeal. It is used for many forest products (McWilliams and Schmidt 1999), and comprises 22 percent of the total volume of softwood growing stock in the northeastern United States (Ward et al. 2004). It is commonly used in ornamental settings, and tree mortality may greatly impact private and public property values (Battles et al. 1999). The economic and environmental impacts of *A. tsugae* will continue to increase as the infestation expands (Quimby 1996); therefore, the preservation of hemlock species is critical due to ecological as well as economic considerations.

Life Cycle of *Adelges tsugae*

In North America, *Adelges tsugae* is anholocyclic, reproducing only asexually on its secondary host, *Tsuga spp.* (McClure 1989). The overwintering generation, or sistens, is present from summer to the following spring. Adult sistens lay 50-300 eggs in woolly ovisacs from March through May (McClure 1989, 1991a). Crawlers emerge in April and May, with nymphs developing into wingless and winged forms. When mature, the winged form, called sexupara, disperses to *Picea spp.* where they lay approximately 12 eggs beneath their folded wings (McClure 1989). The eggs hatch but apparently cannot survive on spruce in eastern North America (McClure 1992b). The proportion of sexuparae produced is directly density-dependent (McClure 1991a). The wingless form, or progrediens, remains on hemlock and deposits an average of 25 eggs per ovisac (McClure 1989, Gray and Salom 1996). Sistens crawlers emerge in June and July, settle at the base of young needles, and immediately enter summer diapause (aestivation) (McClure 1987, Salom et al. 2001). Sistens resume development in October and reach maturity in late winter. Thus, two asexual generations are completed annually on hemlock. The sexual generation has not been observed in western North America (Zilahi-Balogh et al. 2003a), but has been reported in Japan (Inouyue 1945). The life stages and generations of *A. tsugae* in North America often display a great deal of overlap, as phenology varies significantly with elevation, latitude and environmental conditions (McClure et al. 1999).

The specific mechanism by which *A. tsugae* causes mortality in hemlock is unknown, but indirect and direct damage may occur from feeding activities. Adelgid nymphs penetrate plant tissues with their stylet bundle and feed on parenchyma cells in the xylem rays, which serve as nutrient transfer and storage cells (Young et al. 1995). Tree health may be compromised due to the depletion of photosynthates (McClure 1991b), which increases susceptibility to other environmental stressors. A closely related species, the balsam woolly adelgid, *Adelges piceae* Ratz injects toxic saliva during feeding, which leads to high levels of damage at low population densities (Hain 1989). While this has not been confirmed in *A. tsugae*, a similar type of response may occur in hemlock, leading to increased compartmentalization and reduced flow of water and nutrients.

Management of *Adelges tsugae*

In ornamental and nursery settings, systemic insecticides have proven effective in managing *A. tsugae* populations (McClure 1992a, Rhea 1995, Fidgen et al. 2002, Webb et al. 2003); however, forest hemlocks are found in many inaccessible locations or along riparian zones where chemical controls are neither reasonable nor allowed. Since only generalist pathogens have been identified with *A. tsugae* (Reid et al. 2002) and no known parasitoids are associated with Adelgidae (Montgomery and Lyon 1996), biological control efforts using prey-specific predators have been initiated. Recent surveys in the eastern United States indicate that native predators consist primarily of generalists (Wallace and Hain 2000), and that these species do not effectively control *A. tsugae* populations (McClure 1987, Montgomery and Lyon 1996). A classical biological control program is currently being implemented using predators from western North America and Asia (Cheah et al. 2004).

Biological Control of *Adelges tsugae*

Laricobius nigrinus

Laricobius nigrinus Fender (Coleoptera: Derodontidae), native to the western United States and Canada, is a prey-specific predator of *A. tsugae* (Zilahi-Balogh et al. 2002). Derodontidae is a small family comprised of four genera that inhabit the temperate parts of both southern and northern hemispheres, and *Laricobius* is the only genus known to feed on adelgids (Lawrence and Hlavac 1979). *Laricobius nigrinus* is found in western North America on western hemlock *T. heterophylla* (Raf.) Sargent, and this species is not typically injured by *A. tsugae* (Furniss and Carolin 1977). Adults are black, have body lengths of ≤ 3 mm, and feed on all stages of *A. tsugae* (Zilahi-Balogh et al. 2002). Females oviposit singly within *A. tsugae* ovisacs from January to May. Larvae have four instars, during which time body length is increased from 1.7-3.6 mm. Mature larvae migrate to the soil to pupate (Zilahi-Balogh et al. 2003a). This species has good phenological synchrony with *A. tsugae* (Zilahi-Balogh et al. 2003b), as emergent adults remain in the soil in aestival diapause during summer, and resume activity in the fall when *A. tsugae* nymphs begin to develop. Development from egg to adult requires 667 degree-days above a lower developmental threshold of 3.7°C (Zilahi-Balogh et al. 2003c). Field studies indicate that *L. nigrinus* significantly reduces *A. tsugae* densities within temporary branch enclosures, and can survive and reproduce in southwest Virginia from November to April (Lamb et al. 2005a). This species is univoltine and can be mass reared in the laboratory (Lamb et al. 2002). It has been released into hemlock stands in the eastern United States since 2003 (Mausel 2004).

Sasajiscymnus tsugae

Sasajiscymnus (Pseudoscymnus) tsugae Sasaji and McClure (Coleoptera: Coccinellidae), native to Japan, is also a specialist predator of *A. tsugae* (Cheah and McClure 1998, Butin et al. 2002). Adults are black, have body lengths of 1.5-2.3 mm, and feed on all stages of *A. tsugae* (Sasaji and McClure 1997). Females oviposit singly in concealed locations within curled bud-scales, in empty male cones, or beneath *A. tsugae* ovisacs (Cheah and McClure 1998). Larvae have four instars, during which time body length is increased from 1.1-2.7 mm. Mature larvae develop a partial woolly covering, and pupation occurs on the host tree. Development time from egg to adult requires 405 degree-days above a low developmental threshold of 9.5°C (Cheah and McClure 2000). This species has a reproductive diapause that coincides with *A. tsugae* aestivation (Cheah and McClure 2000), and is capable of producing successive generations in the laboratory (McClure and Cheah 1999, Palmer and Sheppard 2002). To date, over 1 million beetles have been released into hemlock stands in the eastern United States, and field collections in Pennsylvania and Connecticut suggest that this predator is established within this region (Cheah et al. 2004).

Harmonia axyridis

In addition to these newly introduced species, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), previously introduced for biological control of various homopteran pests, has quickly spread into many regions of North America (rev. in Koch 2003). Adults are extremely variable in coloration, have body lengths of 4.9-8.2 mm, and are highly polyphagous, voracious predators (Gordon 1985). Females oviposit on host tree substrates in groups of approximately 20-30 eggs (Takahashi 1987). Larvae have four instars, during which time body length is increased from 1.9-10.7 mm, and becomes covered with many spines (Sasaji 1977, Rhoades 1996). Pupation occurs on the host plant of the prey. Development from egg to adult requires 268 degree-days above a low developmental threshold of 11.2°C (Lamana and Miller 1998). This species is bivoltine in North America. Adults migrate to overwintering sites in late fall (Lamana and Miller 1996, Koch and Hutchinson 2003), and in late winter or early spring they switch from diapause to a quiescent state (Iperti and Bertrand 2001). Upon the arrival of warmer temperatures in spring, they mate and disperse from overwintering sites (Lamana and Miller 1996). In the southeastern United States, surveys for natural enemies of *A. tsugae* showed that, while predators were generally scarce overall, *H. axyridis* was the most abundant predator collected from branch beat samples (Wallace and Hain 2000).

Justification for Research

Direct or indirect competitive interactions among species utilizing the same resource at the same time may lead to reductions in predator diversity and decrease the efficacy of biological control (Rosenheim et al. 1993). Direct competition often takes the form of cannibalism or intraguild predation. Relative body size and the degree of trophic specialization are the two most important factors influencing the frequency and direction of these behaviors (Polis et al. 1989). It is usually directed toward species with the greatest temporal or spatial seasonal overlap (Wilson 1971). Although more difficult to detect, indirect competition among may also occur, in which predator activity or behavior is negatively affected as the result of cues, often chemical or tactile (direct contact), from other predators. These interactions can change community structure and ultimately result in the release of the prey population (Rosenheim et al. 1993). Thus, it is essential to determine the nature and effects of intraspecific and interspecific competition so that the establishment and resilience of newly introduced and existing predators are minimally affected.

Competition in terrestrial insect predators often occurs when a large prey population attracts a new predatory species through immigration, or when new predators are intentionally introduced for biological control. In agroecosystem studies, new predator introductions can lead to additive effects that enhance pest suppression (Frazer et al. 1981, Murdoch 1990); however, competition can also destabilize existing complexes and reduce pest suppression (Croft and MacRae 1992, Rosenheim et al. 1993). In forest ecosystems, few studies of competition among predators have been conducted. However, understanding these interactions is crucial when implementing biological control, as the strategy relies on manipulation of the number and diversity of natural enemies.

In the eastern United States, the biological control program for *A. tsugae* includes two specialist predators *L. nigrinus* and *S. tsugae*, which are being released into areas containing primarily native and established exotic generalist predators, including *H. axyridis* (Montgomery and Lyon 1996, Wallace and Hain 2000). Although *H. axyridis* is an important arboreal biological control agent of aphids in pecan (LaRock and Ellington 1996), apple (Brown and Miller 1998) and citrus (Michaud 2002), its expansion into an area can dramatically affect local populations of aphidophagous predators. Declines in the populations of native, arboreal coccinellids, *Brachiacantha ursine* F., *Cycloneda munda* Say and *Chilocorus stigma* Say in southwestern Michigan (Colunga-Garcia and Gage 1998) and *Cycloneda sanguinea* L. in Florida (Michaud 2002), have been associated with invasion by *H. axyridis*. In addition to impacts on native species, *H. axyridis* has also replaced another established exotic species, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), as the predominant predator in arboreal habitats in western Oregon (Lamana and Miller 1996) and West Virginia (Brown and Miller 1998). The mechanisms of replacement are not well understood, but potentially, direct or indirect competitive interference among these species could have altered predator abundance and diversity and affected biological control efforts.

The studies reported in this dissertation examined competitive interactions among two specialist predators of *A. tsugae*, *L. nigrinus* and *S. tsugae,* and an established generalist predator, *H. axyridis* using a series of laboratory and field assays. This represented a first attempt to examine competitive interactions among these species and has provided an indication of the potential for predator compatibility. In addition, our knowledge of many aspects of the biology and ecology of these predators with *A. tsugae* was limited, so predator behavior and daily activity patterns were examined using video studies. The null hypothesis for all studies presented here is that competition among predators will not affect predator responses, while the alternative hypothesis is that competition will negatively impact predator responses and reduce their effectiveness. Taken together, this research provides an increased understanding of the biology and behavioral ecology of these species in the forest ecosystem, and will contribute to the strategies developed and practices carried out in the emerging biological control of *A. tsugae*.

Research Objectives

1. Evaluate competitive interactions among *L. nigrinus, S. tsugae* and *H. axyridis* under simulated natural conditions in the laboratory (Chapter 2).
    a) Examine the susceptibility of predator eggs to cannibalism and interspecific predation and the relationship of these behaviors to *A. tsugae* density.
    b) Evaluate larval and adult female survival, net egg production and feeding on *A. tsugae* in short-term Petri dish assays, and examine the effects of additional conspecific and heterospecific predators on these responses.

2. Evaluate competitive interactions among *L. nigrinus*, *S. tsugae* and *H. axyridis* under natural conditions at field sites in southwestern Virginia (Chapter 3).
    a) Evaluate adult female survival and net reproduction as well as feeding and impact on *A. tsugae* over longer durations in branch cage enclosures.
    b) Examine the effects of additional conspecific and heterospecific predators on these responses.

3. Characterize aspects of the behavior and daily activity of *L. nigrinus*, *S. tsugae* and *H. axyridis* in the laboratory using video recording techniques (Chapter 4).
    a) Describe the searching, feeding, resting and oviposition behavior of each species and document their daily activity patterns.
    b) Examine the effects of additional conspecifics and heterospecifics on temporal and spatial patterns.

Chapter 2

Competitive Interactions Among Predators in Laboratory Assays

Introduction

Hemlock woolly adelgid, *Adelges tsugae* Annand, is a major pest of eastern hemlock, *Tsuga canadensis* L. Carriere, and Carolina hemlock, *T. caroliniana* Engelmann, in the eastern United States. Recent surveys indicate that native predators consist primarily of generalists (Wallace and Hain 2000), and that these species do not effectively control *A. tsugae* populations (McClure 1987, Montgomery and Lyon 1996). A classical biological control program using exotic predators from western North America and Asia is currently underway (Cheah et al. 2004). Two specialist predators, *Laricobius nigrinus* Fender and *Sasajiscymnus tsugae* Sasaji and McClure are currently being released as part of a classical biological control program for *A. tsugae*. In addition, *Harmonia axyridis* Pallas, an aggressive generalist predator, is now commonly found with *A. tsugae* (Wallace and Hain 2000). Direct or indirect competitive interactions among species using the same resource at the same time may lead to reductions in predator diversity and decrease the efficacy of biological control (Rosenheim et al. 1993). In this study, competitive interactions among these species were examined under simulated conditions in laboratory assays. This included examining the susceptibility of predator eggs to cannibalism and interspecific predation in relation to *A. tsugae* density. It also included evaluating larval and adult female survival, net reproduction and feeding on *A. tsugae*, when predators were present alone and when conspecifics or heterospecifics were incorporated into the assay. This represents the first attempt to examine competitive interference among these species, and will provide a preliminary indication of the compatibility of these predators in the field.

Materials and Methods

Insect Cultures

Laricobius nigrinus adults were obtained from a colony at Virginia Polytechnic Institute and State University (Blacksburg, Virginia). *Sasajiscymnus tsugae* adults were obtained from a colony at the Philip Alampi Beneficial Insect Laboratory at the New Jersey Department of Agriculture (Trenton, New Jersey). *Harmonia axyridis* adults were obtained from Rincon-Vitova Insectaries (Ventura, California). All predators were reared under their normal developmental conditions at 10°C, LD 12:12 h, 75% RH for *L. nigrinus* (Lamb et al. 2005b), and at 25°C, LD 16:8 h, 45% RH for both coccinellid predators (Palmer and Sheppard 2002, Matsuka and Niijima 1985). All predator species were placed in 2.2 L plastic containers lined with moistened filter paper and ventilated with fine polyester mesh (Sefar). Each container held 15 adults, in a sex ratio of 2F:1M and 5-7 *T. canadensis* branch clippings heavily infested with *A. tsugae*. Branches were removed weekly and placed into containers with fresh, infested *T. canadensis* clippings to facilitate larval development. Containers were maintained in an environmental chamber (Percival-Scientific).

Branch clippings were removed as needed and searched to obtain appropriate predator life stages for the experimental assays. Adult predators were mature, of approximately the same age (12-24 wk), and were selected randomly from rearing containers. Sex of predators was identified using morphological characters of *H. axyridis* (Gordon 1985) and *S. tsugae* (Sasaji and McClure 1997) and by monitoring oviposition by *L. nigrinus*. Fourth-instar *L. nigrinus* and second-instar *S. tsugae* and *H. axyridis* were used for all larval trials in accordance with the predicted overlap of predator life stages in hemlock stands, based on temperature development requirements (Cheah and McClure 1998, LaMana and Miller 1998, Zilahi-Balogh et al. 2003c). Predator instars were determined using head capsule and body length measurements of *L. nigrinus* (Zilahi-Balogh et al. 2003a), *S. tsugae* (Cheah and McClure 1998) and *H. axyridis* (Rhoades 1996).

Experimental Design of Laboratory Studies

All experimental assays consisted of 15 x 2.5 cm polystyrene Petri dishes (Fisher-Scientific) lined with moistened filter paper (Figure 2.1). Prior to laboratory assays, a subset of predators was moved to new containers and temperature and photoperiod were gradually modified over 2-3 wk to precondition predators to experimental conditions. Two evaluation periods were used, based on temperature and RH averages obtained by data loggers (Onset Computers) placed in hemlock stands in southwestern Virginia from March 1-April 15 and April 16-May 31, 2003. These are termed 1) early spring: DN 10:5˚C, LD 12:12 h, 50-75% RH, and 2) late spring: DN 20:10˚C, LD 16:8 h and 65-85% RH. These time periods cover the approximate duration of *A. tsugae* adult sistens activity (McClure 1989). Unless otherwise indicated, all larval assays were tested using only the late spring conditions, while adult assays were tested at the each set of conditions in keeping with the longer duration of adult activity. Except where indicated, all larval assays contained 20 cm of *T. canadensis* branch clippings with 50 *A. tsugae* adult sistens with ovisacs. Adult predator assays contained 60 cm of branch clippings with 150 *A. tsugae* adult sistens with ovisacs. In each assay, measurements of predator survival, feeding and egg production were assessed simultaneously at the conclusion of the trial. Fifteen replicates of each experiment were completed.

Figure 2.1. Experimental assay for the laboratory studies. Each assay consisted of a 15 x 2.5 cm polystyrene Petri dish lined with moistened filter paper, and held 20-60 cm of branch clippings of eastern hemlock, *T. canadensis*, infested with *A. tsugae* adult sistens or progrediens with ovisacs.

Predator Feeding on Predator Eggs

No-choice tests were used to examine predator feeding on conspecific and heterospecific eggs at two predator life stages and three *A. tsugae* densities. Each assay held either one larva or one adult female predator and 10 cm of *T. canadensis* with 0, 5 or 10 *A. tsugae* adult sistens with ovisacs. Each predator was also provided with three predator eggs.

The eggs were moved into the assay from natural oviposition locations using a fine-tip brush, and only one egg species was tested at a time. Eggs of *L. nigrinus* were placed singly into *A. tsugae* ovisacs, eggs of *S. tsugae* were placed singly on bud scales, and *H. axyridis* eggs were placed in an exposed group on a single host needle. These locations are consistent with the typical oviposition sites of *L. nigrinus* (Zilahi-Balogh et al. 2003a), *S. tsugae* (Cheah and McClure 1998) and *H. axyridis* (Takahashi 1987). During egg removal from their original location, attempts were also made to preserve and relocate materials used by females to attach eggs to the substrate. Under natural conditions, *H. axyridis* often lays more than three eggs per site; however, egg density was standardized for all species in these assays so that predation comparisons could be made. Assays were tested using the late spring conditions, and the number of conspecific or heterospecific predator eggs eaten by each individual predator was counted after 48 h. The consumption of predator eggs was analyzed separately for larvae and adults of each species using general linear models (PROC GLM, SAS Institute 2001) with predator species, predator egg species and their interaction as categorical independent variables, and *A. tsugae* density as a covariate. Simple linear regression was used to examine the relationship between predator egg consumption and *A. tsugae* density for each predator feeding on each egg species (Zar 1998).

Predator Survival

Larval and adult predator survival was assessed for each species alone and when grouped with two additional conspecific or heterospecific predators. In larval assays, two different life stages were used, which consisted of larvae of the appropriate instar, or adult females. Adult assays were evaluated in two environments that corresponded to early and late spring conditions. In conspecific groupings, survival was recorded for one larva or adult female, selected at random, which were marked prior to the study. Survival was recorded as a binomial response: alive=1, dead=0 after 6 d.

Predator Net Egg Production and Feeding on *A. tsugae*

The effects of conspecifics and heterospecifics on predator net egg production and feeding on *A. tsugae* sistens was assessed using microscopic inspection of branch clippings after 6 d. Net egg production was determined using counts of all intact predator eggs. For conspecific treatments, the net egg production per female predator was calculated. Eggs were distinguished using morphological characteristics of *L. nigrinus* (Zilahi-Balogh et al. 2003a), *S. tsugae* (Sasaji and McClure 1997) and *H. axyridis* (Gordon 1985). Larval feeding measures were determined using the number of *A. tsugae* sistens ovisacs ≥ 50% eaten. The approximate number of eggs per *A. tsugae* ovisac present initially was determined *a priori* by averaging the results of 4 undisturbed ovisacs taken from each assay. The approximate number of eggs remaining in disturbed ovisacs at the conclusion of each trial were counted and subtracted from the *a priori* mean. Adult female feeding was determined by counts of the number of sistens ovisacs with evidence of predation; that is, when the adelgid ovisac was disrupted and the adult or progeny within showed evidence of being fed upon or killed. Adult feeding was distinguished from other causes of mortality by the presence of puncture wounds or damage to the exoskeleton that was consistent with previously observed attacks. Predation on *A. tsugae* in each combination was assessed as a total for all predators in the treatment, as feeding by individual species could not be distinguished.

Intra- and interspecific interference were assessed by comparing the responses of individual larvae or females of each species to that of the average or combined responses of their respective conspecific grouping and all heterospecific groupings that contained the species being evaluated. For net reproduction, in the absence of significant competitive interference, we would expect similar responses from individual females of each species and that of the heterospecific groupings containing that species. The average net egg production per female, in conspecific groupings, should also be similar to these values. Differences among the treatments were used to indicate significant predator interference. In contrast, in the absence of significant competitive interference, we would expect total predator feeding to be greater in conspecific and heterospecific groupings than in treatments with individual larvae or adult females. Similarity among the treatments for this response was used to indicate significant predator interference.

Statistical Analyses

Predator survival was analyzed separately for larvae and adult females using logistic regression (PROC LOGISTIC, SAS Institute 2001), while predator egg production and feeding responses were analyzed using general linear models (PROC GLM, SAS Institute 2001). In each case, the model included predator species, predator species combination, and either predator life stage combination (larvae) or environmental condition (adult females), and their interactions as independent categorical variables. Results for net egg production and feeding responses were $\log_{10}$ transformed to achieve normality and equality of variances, and analyzed using two-way analysis of variance (Zar 1998). All analyses of variance were followed by Tukey's honestly significant difference test to separate treatment means (Zar 1998), and results were evaluated for significance at $P \leq 0.05$.

Results

Predator Feeding on Predator Eggs

The model for predators feeding on predator eggs had a strong interaction between predator species and predator egg species in both larval ($F = 22.54$; df = 4,125; $P \leq 0.0001$) and adult trials ($F = 16.37$; df = 4,125; $P \leq 0.0001$), so each species was analyzed separately. *Laricobius nigrinus* consumed significantly more conspecific eggs than heterospecific eggs, and significantly fewer *H. axyridis* eggs as both larvae ($F = 29.91$; df = 2,41; $P \leq 0.0001$) and adult females ($F = 21.26$; df = 2,41; $P \leq 0.0001$). For predator larvae, there was a negative relationship between consumption rates of predator eggs and increasing *A. tsugae* density in assays with eggs of conspecifics ($r^2 = 0.66$; $P \leq 0.0001$) and *S. tsugae* ($r^2 = 0.67$; $P \leq 0.0001$), but not with *H. axyridis* eggs ($r^2 = 0.10$; $P = 0.24$) (Figure 2.2.A). These relationships were consistent for adult females in assays with eggs of conspecifics ($r^2 = 0.58$; $P = 0.0010$), *S. tsugae* ($r^2 = 0.62$; $P = 0.0004$) and *H. axyridis* ($r^2 = 0.10$; $P = 0.24$) (Figure 2.3.A).

Sasajiscymnus tsugae consumed similar numbers of conspecific and *L. nigrinus* eggs, but significantly fewer eggs of *H. axyridis* in both larval ($F = 5.93$; df = 2,41; $P = 0.02$) and adult female ($F = 9.60$; df = 2,41; $P \leq 0.0001$) trials. Similar to *L. nigrinus*, there was a negative relationship between consumption rates of predator eggs and increasing *A. tsugae* density for larvae in assays with eggs of conspecifics ($r^2 = 0.59$; $P \leq 0.0001$) and *L. nigrinus*

($r^2 = 0.28$; $P = 0.02$), but not with *H. axyridis* ($r^2 = 0.10$; $P = 0.24$) (Figure 2.2.B). These relationships were maintained for adult female predation on eggs of conspecifics ($r^2 = 0.59$; $P \leq 0.01$), *L. nigrinus* ($r^2 = 0.49$; $P \leq 0.001$) and *H. axyridis* ($r^2 = 0.10$; $P = 0.24$) (Figure 2.3.B).

Harmonia axyridis showed significantly greater feeding on conspecific eggs than heterospecific eggs, which each had similar amounts of predation in larval ($F = 16.58$; df = 2,41; $P \leq 0.01$) and adult female ($F = 16.58$; df = 2,41; $P \leq 0.0001$) trials. Larvae of this species demonstrated a negative relationship between feeding on predator eggs and increasing *A. tsugae* density in assays with eggs of *L. nigrinus* ($r^2 = 0.61$; $P \leq 0.0001$) and *S. tsugae* ($r^2 = 0.78$; $P \leq 0.0001$), but not with conspecific eggs ($r^2 = 0.08$; $P = 0.34$) (Figure 2.2.C). These relationships were maintained for adult female egg predation of *L. nigrinus* ($r^2 = 0.69$; $P \leq 0.01$), *S. tsugae* ($r^2 = 0.62$; $P = 0.0004$) and conspecifics ($r^2 = 0.09$; $P = 0.29$) (Figure 2.3.C).

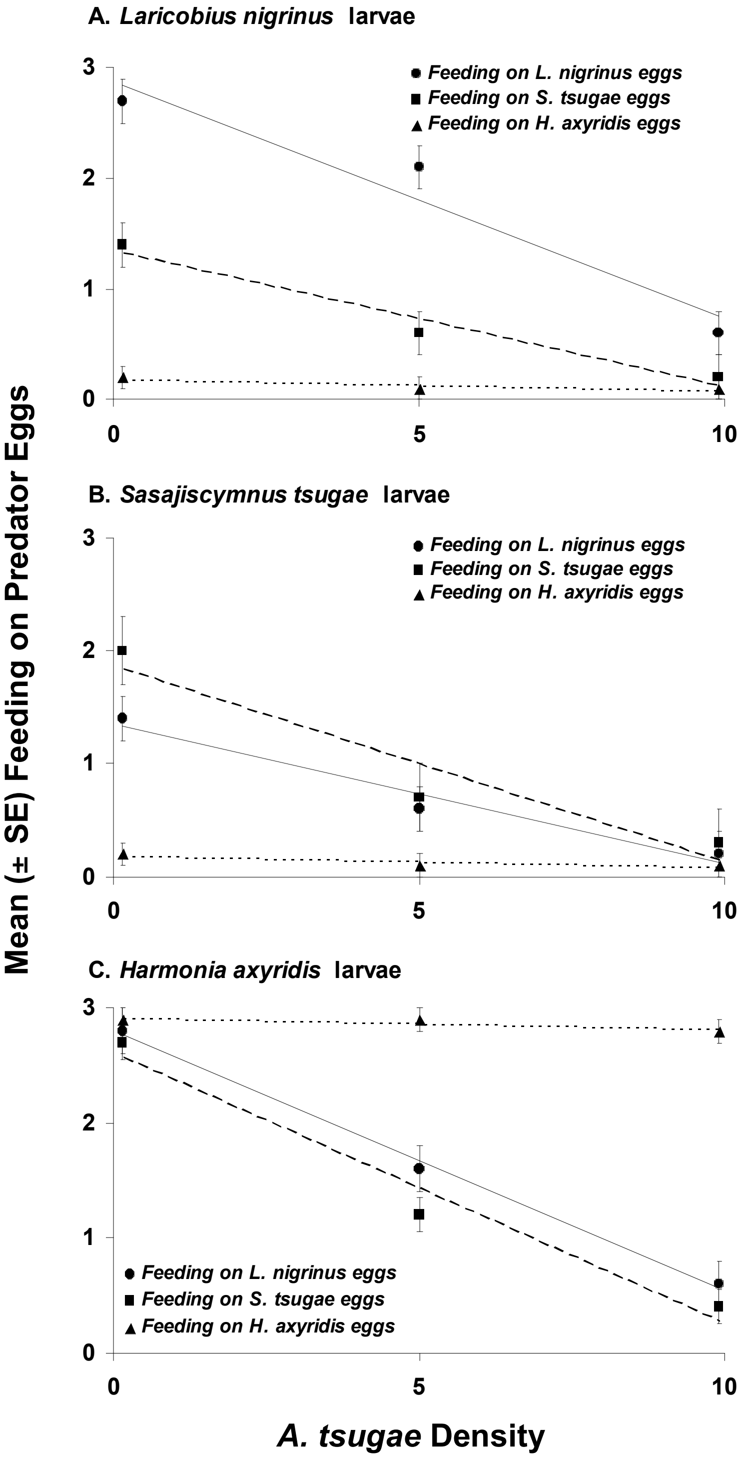

Figure 2.2. Mean feeding (± SE) on predator eggs of each species by larvae of (A) *L. nigrinus*, (B) *S. tsugae* and (C) *H. axyridis* after 48 h, and the relationship between predator egg consumption and *A. tsugae* density (*n*=15).

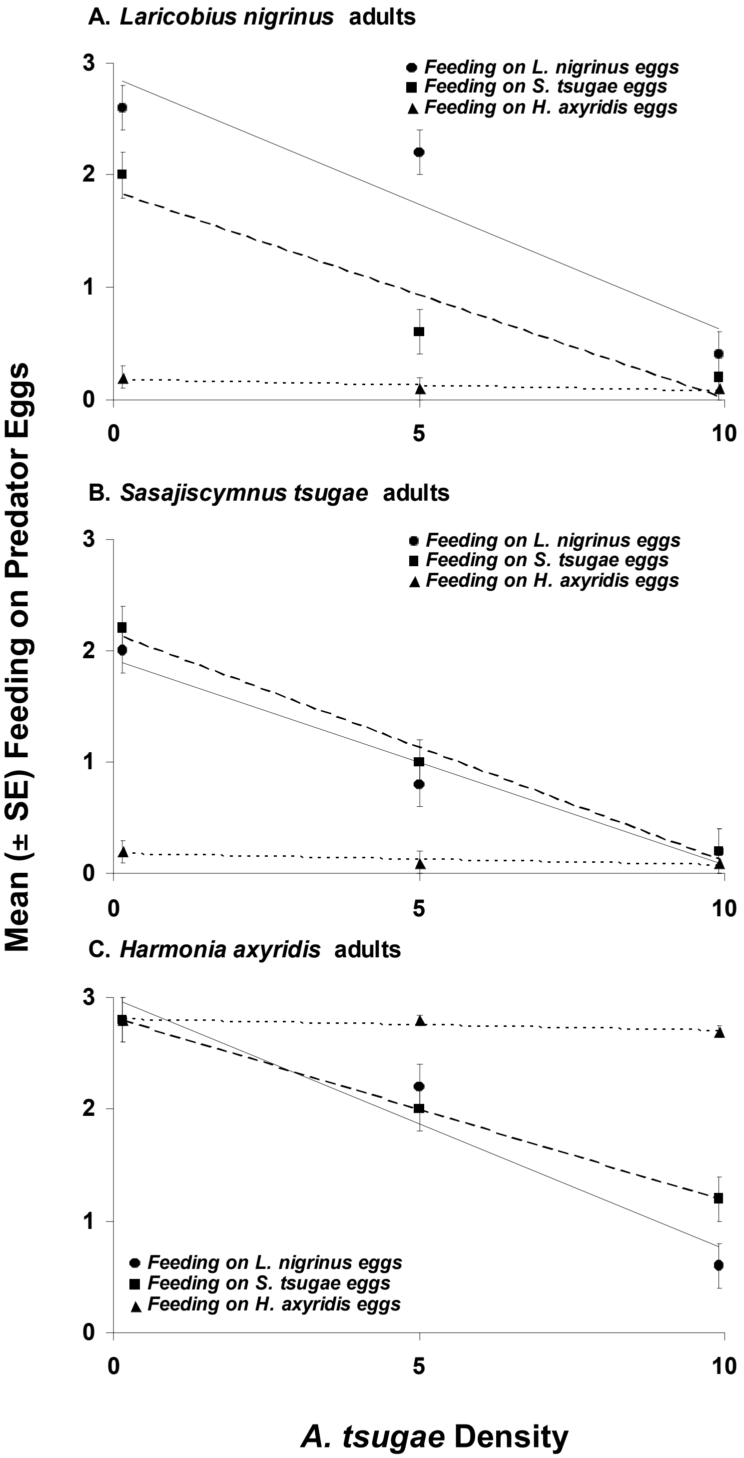

Figure 2.3. Mean feeding (± SE) on predator eggs of each species by adult females of (A) *L. nigrinus*, (B) *S. tsugae* and (C) *H. axyridis* after 48 h, and the relationship between predator egg consumption and *A. tsugae* density (*n*=15).

Predator Survival

Survival of predators was high overall and ranged between 71.4 and 92.3%. There was no interaction between the model variables for larvae of *L. nigrinus* (Wald χ^2 = 0.23; d.f. = 2; *P* = 0.62), *S. tsugae* (Wald χ^2 = 0.40; d.f. = 2; *P* = 0.82) and *H. axyridis* (Wald χ^2 = 0.26; d.f. = 2; *P* = 0.88) or for adult females of *L. nigrinus* (Wald χ^2 = 0.11; d.f. = 2; *P* = 0.95), *S. tsugae* (Wald χ^2 = 1.35; d.f. = 2; *P* = 0.51) and *H. axyridis* (Wald χ^2 = 0.12; d.f. = 2; *P* = 0.92). Therefore, each factor was evaluated across all other variable levels. For both larval and adult trials, there were no significant differences in survival related to predator species combination (Table 2.1). In larval trials, there were also no significant differences by predator life stage combination (larvae or adults) for *L. nigrinus* (Wald χ^2 = 0.49; d.f. = 1; *P* = 0.48), *S. tsugae* (Wald χ^2 = 0.46; d.f. = 1; *P* = 0.45), and *H. axyridis* (Wald χ^2 = 2.14; d.f. = 1; *P* = 0.15). Similarly, adult female survival was not different by environment (early or late spring) for *L. nigrinus* (Wald χ^2 = 0.05; d.f. = 1; *P* = 0.83), *S. tsugae* (Wald χ^2 = 0.06; d.f. = 1; *P* = 0.81) or *H. axyridis* (Wald χ^2 = 0.004; d.f. =1; *P* = 0.98.)

Table 2.1. Percent survival of larval and adult female predators in Petri dish assays with *A. tsugae* after 6 d. Predators were evaluated alone and in combination with two conspecifics and two heterospecifics at two simulated environments (early and late spring). *There were no significant differences by predator combination for any species ($P \geq 0.05$).

Life Stage (Environment)	Predator Species[1]	Predator Combination[1]	*n*	Survival (%)	Wald $\chi 2$	**P*
Larvae (Early Spring)	*Ln*	Alone	25	80.0	0.31	0.86
		Ln+Ln+Ln	24	79.2		
		Ln+St+Ha	26	73.1		
	St	Alone	24	75.0	0.16	0.92
		St+St+St	27	81.5		
		St+Ln+Ha	26	73.1		
	Ha	Alone	27	85.2	1.68	0.43
		Ha+Ha+Ha	28	71.4		
		Ha+Ln+St	26	84.6		
Adults (Early and Late Spring)	*Ln*	Alone	27	81.5	0.35	0.81
		Ln+Ln+Ln	26	76.9		
		Ln+St+Ha	25	84.0		
	St	Alone	27	85.1	0.21	0.90
		St+St+St	28	78.6		
		St+Ln+Ha	25	72.0		
	Ha	Alone	26	92.3	0.80	0.98
		Ha+Ha+Ha	28	89.3		
		Ha+Ln+St	25	92.0		

[1] *Ln*: *Laricobius nigrinus, St: Sasajiscymnus tsugae, Ha: Harmonia axyridis*

Predator Net Egg Production

Analyses of predator reproduction had no interaction between the variables for *L. nigrinus* (*F* = 2.44; d.f. = 2,84; *P* = 0.13), *S. tsugae* (*F* = 0.48; d.f. = 2,84; *P* = 0.62) or *H. axyridis* (*F* = 0.53; d.f. = 2,84; *P* = 0.59), so each factor was evaluated across the other variable levels. There were significant differences by predator species combination for *L. nigrinus* (*F* = 57.68; d.f. = 2,84; *P* ≤ 0.0001) (Figure 2.4.A) and *H. axyridis* (*F* = 5.51; d.f. = 2,84; *P* = 0.006) (Figure 2.4.C), but not *S. tsugae* (*F* = 0.44; d.f. = 2,84; *P* = 0.65) (Figure 2.4.B). The highest net egg production was by *L. nigrinus*, while *S. tsugae* and *H. axyridis* each had relatively low levels by comparison. All species had lower net reproduction per predator in conspecific groupings; however, it was only reduced to a statistically significant degree for *L. nigrinus* and *H. axyridis* (Figure 2.4.A-C). Heterospecific groupings did not significantly affect net reproduction by these species. Net reproduction by evaluation period was greater for all predators in late spring: *L. nigrinus* (*F* = 37.58; d.f. = 1,84; *P* ≤ 0.0001), *S. tsugae* (*F* = 64.45; d.f. = 1,84; *P* ≤ 0.0001) and *H. axyridis* (*F* = 5.65; d.f. = 1,84; *P* = 0.019).

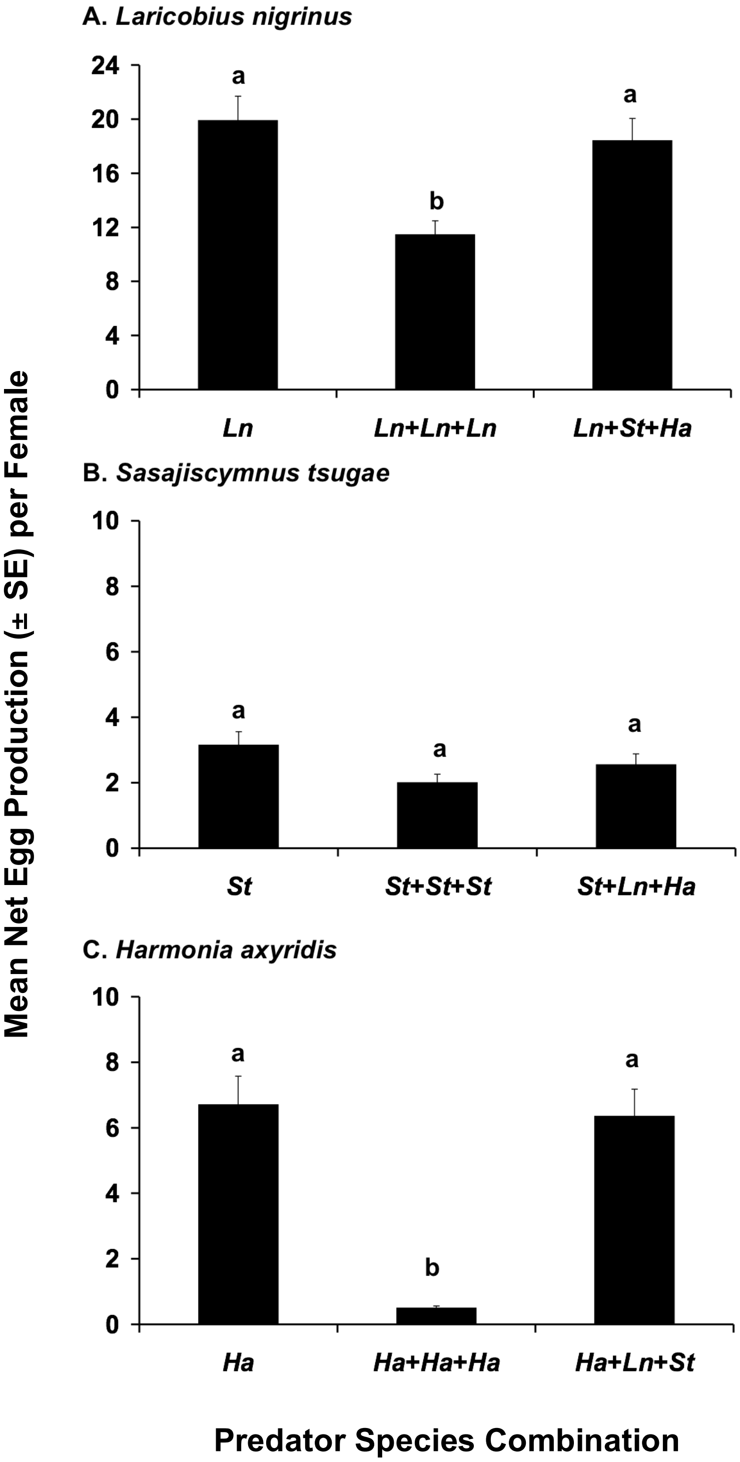

Figure 2.4. Mean net egg production (± SE) per female predator, by species combination, for (A) *L. nigrinus* (*Ln*), (B) *S. tsugae* (*St*) and (C) *H. axyridis* (*Ha*) after 6 d. Each species was evaluated alone and in combination with two conspecific and two heterospecific predators (*n*=30). Means with the same letter were not significantly different ($P \geq 0.05$).

Predator Feeding on *A. tsugae*

For predator feeding on *A. tsugae*, there were again no interactions between model variables for larvae (F = 3.14; d.f. = 6,196; P = 0.065) or adult females (F = 2.51; d.f. = 6,196; P = 0.089), so each factor was evaluated across other variable levels. Feeding was significantly different by predator species combination for larvae (F = 325.2; d.f. = 6,196; $P \leq$ 0.0001) (Figure 2.5.A) and adult females (F = 292.1; d.f. = 6,196; $P \leq$ 0.0001) (Figure 2.5.B). In larval trials, individual species comparisons showed that *H. axyridis* and *L. nigrinus* fed on significantly more sistens than *S. tsugae*. In adult trials, *H. axyridis* had a significantly greater amount of feeding than *L. nigrinus* or *S. tsugae*. For larvae and adult females, comparisons of individual predator species, with their respective conspecific and heterospecific groupings revealed that total feeding was significantly higher for all species when placed in predator groupings. For larvae, the greatest amount of feeding occurred in conspecific groupings of *L. nigrinus* and *H. axyridis*, while the conspecific grouping of *H. axyridis* had the highest amount of feeding in adult female trials. Analyses by life stage combination for predator larvae indicated that there was significantly more feeding with adults (F = 236.6; d.f. = 1,196; $P \leq$ 0.0001). Analyses by environment for adult females indicated that there was significantly more feeding in late spring (F = 163.9; d.f. = 1,196; $P \leq$ 0.0001).

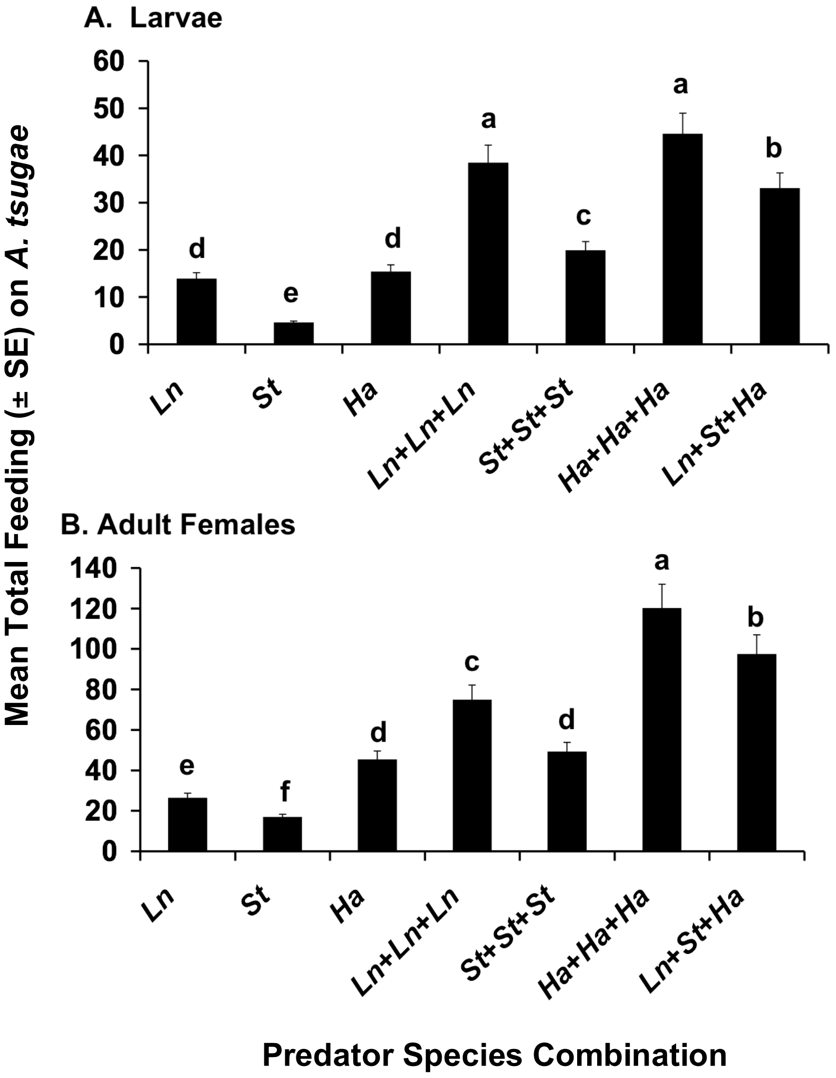

Figure 2.5. Mean total feeding (± SE) on *A. tsugae* sistens by (A) larvae and (B) adult females of *L. nigrinus* (*Ln*), *S. tsugae* (*St*) and *H. axyridis* (*Ha*) after 6 d. Feeding corresponded to the number of *A. tsugae* adults and/or ovisacs that showed evidence of disruption and predation. Each species was evaluated alone and in combination with two conspecific and two heterospecific predators (*n*=30). Means with the same letter were not significantly different ($P \geq 0.05$).

Discussion

Among the specialist species, differences in predator egg susceptibility to predation may be explained as a function of predator feeding specificity and reproductive biology. The narrow prey range of *L. nigrinus* (Zilahi-Balogh et al. 2002) and *S. tsugae* (Cheah and McClure 1998, Butin et al. 2002) severely limited predation except in cases where no *A. tsugae* was provided. Although eggs of these species were vulnerable to predation, the inverse relationship that existed between predator egg consumption and *A. tsugae* density likely arose from a decline in the probability of encountering predator eggs as *A. tsugae* density increased, suggesting that eggs of these species are being preyed upon opportunistically rather than preferentially. Eggs of *L. nigrinus* were also oviposited within *A. tsugae* ovisacs, and may have suffered higher levels of predation than *S. tsugae* eggs, which were located on branch substrates. Eggs of the specialist species were fed upon more heavily by conspecifics, indicating that resource competition occurs in these species and that they have developed the ability to cannibalize. Feeding by the specialists on *H. axyridis* eggs was rare, and this may be attributed to the presence of chemical defenses exhibited by polyphagous coccinellid species (Pasteels et al. 1973). However, despite protection from the specialist species, *H. axyridis* egg*s* were consumed at very high rates by conspecifics. This behavior has been shown to provide increased nutrition, growth and survival when prey is of low quantity or quality (Osawa 1992, Wagner et al. 1999, Yasuda and Ohnuma 1999, Snyder et al. 2000, Michaud and Grant 2004).

In these assays, all species had high rates of survival overall, due in part to the brevity of the trials and the high *A. tsugae* densities provided. Previous observations of *L. nigrinus*

(Lamb et al. 2002), *S. tsugae* (Blumenthal 2002) and *H. axyridis* (Osawa 1992, Cottrell and Yeargan 1998, Casagrande et al. 2002) indicate that larval cannibalism can occur; however, prey densities were high throughout the trial and may have limited intraspecific interference by direct means. In addition, the chemical ecology (Pasteels et al. 1973) and defensive spines of *H. axyridis* larvae (Dixon 2000) may have lowered its susceptibility to predation. Interspecific predation has been previously documented in *H. axyridis* larvae and adults (rev. in Koch 2003) when prey is of low quality or quantity, but this did not occur to a significant degree in these assays. For larvae, this may have been limited by the early life stages of *H. axyridis* used, while temperatures for adult females were sub-optimal and may have reduced predator activity.

Although *L. nigrinus* had the highest net reproduction in these trials, other studies have shown higher mean fecundity for *S. tsugae* (Cheah and McClure 1998) and *H. axyridis* (Stathas et al. 2001) over the same ovipositional period. However, the temperature requirements are also much greater for these latter two species. The developmental threshold for *L. nigrinus* is lower than that of *S. tsugae* or *H. axyridis* by 5.8 and 7.5ºC, respectively (Cheah and McClure 1998, LaMana and Miller 1998, Zilahi-Balogh et al. 2003b). Because egg production by all species is influenced by temperature (Cheah and McClure 1998, Stathas et al. 2001, Zilahi-Balogh et al. 2002), the lower threshold of *L. nigrinus* may have allowed increased reproduction at the low temperatures used in these assays. Temperatures and photoperiods during the late spring evaluation were similar to optimal rearing temperatures for *S. tsugae* and *H. axyridis*; however, reproduction was still limited, possibly due to inadequate prey quality (Palmer and Sheppard 2002), host tree health (Sheppard and Palmer

2004) or nutrient limitation. Additionally, the Petri dish enclosure limited predator dispersal and choice of potentially more favorable microhabitats.

Reproductive interference by conspecifics was evident to varying degrees. Negative effects were most apparent for *H. axyridis* and *L. nigrinus*, while *S. tsugae* displayed a lesser degree of interference. Previous studies of *H. axyridis* indicate that indirect reproductive interference occurs by passive substrate marking using fecal cues (Agarwala et al. 2003) and oviposition-deterring pheromones (Yasuda et al. 2000). Direct interference by egg cannibalism also occurs (Osawa 1992), and provides increased nutrition and growth when prey is of low quantity or quality (Wagner et al. 1999, Yasuda and Ohnuma 1999, Snyder et al. 2000, Michaud and Grant 2004). In these studies, both direct and indirect competition was apparent. Microscopic examination of branch clippings showed remnant chorions of *H. axyridis* eggs due to cannibalism. However, it appeared that oviposition did not occur initially in most cases, and that reproductive interference for *H. axyridis* was by indirect means. The chemical ecology exhibited by this species has not been investigated for the specialist predators, but similar mechanisms may be used. In contrast, heterospecific reproductive interference did not occur to a significant degree. Although predation of heterospecific eggs by *H. axyridis* is well documented (Yasuda and Shinya 1997, Burgio et al. 2002), it may have been limited in these studies due to high host densities and low temperatures. In addition, chemical deterrents (Pasteels et al. 1973) may have prevented interspecific predation of *H. axyridis* eggs. Net reproduction was greater in the late spring conditions, suggesting that environmental conditions are the limiting factor for reproduction by these species.

Feeding by predators was greatly influenced by relative body size and experimental temperatures. Late instar *L. nigrinus* and early instar *H. axyridis*, were of similar size (3-4 mm) and larger than early instar *S. tsugae* (1-2 mm), and had greater feeding accordingly. Larval feeding on *A. tsugae* was greater when combined with adult predators than with other larvae. This is due to the larger size of adult predators, and their feeding modality that disrupts adelgid ovisacs and may have facilitated larval entry. Feeding in the adult female trials showed similar patterns. The larger and more voracious *H. axyridis* exhibited a greater amount of feeding than the smaller specialist species. Although these latter two species are similar in body size, higher feeding by *L. nigrinus* is again related to a lower temperature threshold (Zilahi-Balogh et al. 2003b, Lamb et al. 2005a). Feeding by all species was greater in late spring, supporting studies that show these predators are more active at increased temperatures (Lamana and Miller 1998, Cheah and McClure 2000, Zilahi-Balogh et al. 2003b). The incorporation of additional conspecific and heterospecific predators for larvae and adult females significantly increased their total amount of feeding. It was not possible to determine if per predator feeding average increased (synergistic effects), but it does indicate that predator interference did not occur to such a degree that negative effects on feeding activity occurred.

Competitive interactions among predators may directly or indirectly affect predator diversity and biological control (Rosenheim et al. 1993, Polis et al. 1989, Lucas et al. 1998). Initial laboratory studies of these three predators of *A. tsugae* indicate that conspecific interference may negatively affect egg production in these species, while heterospecific interference was limited. These results do not provide definitive conclusions as to the level of competition that may occur among these species under different ecological conditions or longer durations. The experimental design has many constraints, as Petri dish assays in the laboratory may not accurately reflect field conditions or the potential for predator immigration and emigration in response to prey abundance or quality. In addition, the level of competition among predators may have been constrained by the reduced activity of the coccinellid species at low temperature experimental conditions. However, the outcomes of the interactions described in these studies are likely to be representative of what would occur in the field.

Chapter 3

Field Evaluation of Competitive Interactions Among Predators

Introduction

Recent surveys in the eastern United States indicate that native predators of hemlock woolly adelgid, *Adelges tsugae* Annand, consist primarily of generalists (Wallace and Hain 2000), and that these species do not effectively control *A. tsugae* populations (McClure 1987, Montgomery and Lyon 1996). Therefore, two exotic specialist predators, *Laricobius nigrinus* Fender from western North America and *Sasajiscymnus tsugae* Sasaji and McClure from Japan, are currently being released as part of a classical biological control program for *A. tsugae*. In addition, *Harmonia axyridis* Pallas, an aggressive generalist predator, is commonly found with *A. tsugae* (Wallace and Hain 2000). Direct or indirect competitive interactions among species using the same resource may lead to reductions in predator diversity and decrease the efficacy of biological control (Rosenheim et al. 1993). In previous laboratory studies (Chapter 2), there were varying degrees of conspecific interference among these species, while heterospecifics showed non-interference. However, these experiments had rather limited scale and duration. In this study, competitive interactions among these predators were examined over longer durations and under more natural conditions using branch cage enclosures of infested hemlocks in southwestern Virginia. This included evaluating adult female survival, net reproduction, feeding and impact on *A. tsugae*, and examining the effects of additional conspecific and heterospecific predators on these responses.

Materials and Methods

Insect Cultures

Laricobius nigrinus adults were obtained from a colony reared at Virginia Polytechnic Institute and State University (Blacksburg, Virginia). *Sasajiscymnus tsugae* adults were obtained from a colony at the Philip Alampi Beneficial Insect Laboratory at the New Jersey Department of Agriculture (Trenton, New Jersey). *Harmonia axyridis* adults were obtained from Rincon-Vitova Insectaries (Ventura, California). All predators were reared under their normal developmental conditions at 10°C, LD 12:12 h, 75% RH for *L. nigrinus* (Lamb et al. 2005b), and at 25°C, LD 16:8 h, 45% RH for both coccinellid predators (Palmer and Sheppard 2002, Matsuka and Niijima 1985). The coccinellid species were stored at 20°C, LD 16:8 h, 45% RH before shipment to our laboratory (Palmer and Sheppard 2002). Upon receiving the predators, conditions were gradually stepped down from 20 to 10°C and from LD 16:8 to LD 12:12 over a 2-3 wk period to precondition predators to existing field conditions. For preconditioning, all species were placed in 2.2 L plastic containers lined with moistened filter paper and ventilated with fine polyester mesh (Sefar). Each container held 15 adults (ratio of 2F:1M) and 5-7 *T. canadensis* branch clippings heavily infested with *A. tsugae*. Containers were maintained in an environmental chamber (Percival-Scientific). Adult predators were removed from holding containers 24 h before deployment to the field, and placed individually, along with branch clippings containing 10 *A. tsugae*, into 1 x 5 cm Petri dishes (Fisher-Scientific) lined with moistened filter paper. All predators were mature, of approximately the same age (12-24 wk), and were selected randomly from rearing containers. The sex of predators was identified using morphological characters for *H. axyridis* (Gordon 1985) and *S. tsugae* (Sasaji and McClure 1997) and by monitoring oviposition by *L. nigrinus*. During preconditioning, egg production by all species was evident in holding containers. Microscopic examination of a subset of the *A. tsugae* infested foliage that predators were placed on, before deployment in the field, showed that predator eggs of each species were present. This confirmed that some females were reproductively active, and suggested that reproductive capabilities were not impaired by preconditioning, even though it was not possible to insure this for each female.

Experimental Design of Field Studies

Experiments were conducted during two seasons, spring and summer, in both 2003 and 2004 in natural hemlock stands in southwestern Virginia. *Adelges tsugae* sistens adults were present during the spring evaluation and progrediens adults were present during the summer evaluation. In 2003, predators were tested at a site in Jefferson National Forest (37° 23.17'N, 80° 33.83'W; Elev. 925 m). Due to the rapid decline of hemlock health in this area, a different field site was chosen in 2004 in the Mt. Rogers National Recreation Area (36° 45.94'N, 81° 18.17'W; Elev. 780 m). In 2003, previously mated female predators were used. The low level of reproduction by the coccinellid species suggested that females may not have been sufficiently mated prior to introduction to field enclosures or may require multiple-mating throughout the season to insure reproductive success. Therefore, in 2004, sexual pairs of predators were used. In each season, each predator species was evaluated alone and in conspecific and heterospecific groupings. Conspecific groupings contained two females (2003) or two sexual pairs (2004) of the same species. Heterospecific groupings included two- and three-predator species combinations of females or sexual pairs in all possible combinations. All species were tested during the spring; however, *L. nigrinus* was excluded from the summer evaluation due to its natural aestivation period (Zilahi-Balogh et al. 2003b).

At each study site, during each season, 15 *T. canadensis*, with 5-10 branches heavily infested with *A. tsugae*, were selected. Trees were of similar age (20-40 yr), based on tree core samples, and health; each had 3-6 cm of new growth per shoot. Branches were selected on all sides of the tree and standardized by height above the ground (1-2 m), size (0.3 x 0.6 m), and *A. tsugae* density (≥ 3 *A. tsugae*/cm). Branch densities of *A. tsugae* were obtained 48 h before each evaluation by counting the number of woolly masses of sistens in the spring and progrediens in the summer. Intact woolly masses, possessing clear honeydew, and with no apparent damage from predators, were considered to be alive. A completely randomized design was used during each season and at each site, and branches were assigned to either a predator treatment or a no-predator control. The terminal and basal area of the branches were pruned and a fine-mesh polyester fabric cage (0.5 x 1 m) was placed over the foliage (Figure 3.1). Each enclosed branch area contained 250-300 cm of infested foliage that held approximately 750-1000 sistens adults in spring and 500-750 progrediens adults in summer. Prey densities are quite high in natural infestations, and this experimental design accurately represents the conditions under which interactions are likely to occur in the field. Attempts were made to remove all native predators from branches prior to cage placement, and all treatment groups were introduced at the same time into the enclosures. After predator introduction, cages were sealed at the base using foam wrapping and cinch ties. Control branches were evaluated with and without branch cages.

Figure 3.1. Hemlock branch enclosures for field studies. Each enclosure consisted of a 0.5 x 1 m fine-mesh polyester fabric cage, and held 250-300 cm of eastern hemlock, *T. canadensis*, infested with *A. tsugae* adult sistens or progrediens with ovisacs.

Predators remained in the enclosures for 6 wk during the spring (April 1-May 14) and 4 wk during the summer (June 1-July 1). This experimental duration represented the time frame when successful predator reproduction on *A. tsugae* could occur. This is due to the availability of *A. tsugae* eggs, which are essential for survival and growth of predator progeny. In late summer, first instar sistens are in aestivation, an inappropriate stage for predator progeny development. Nine replications of each treatment and control were completed in 2003, and 10 replications were completed in 2004. Two data loggers (Onset

Computer) were used to record stand conditions. Mean temperature and RH in 2003 were 10±2°C, 60-70% RH during the spring and 19±3°C, 70-80% RH during the summer. In 2004, stand conditions were 12±2°C, 65-75% RH and 24±3°C, 75-85% RH, during the two evaluation periods, respectively. At the conclusion of each study, branches were cut and returned intact to the laboratory. The number of surviving adult and immature predator life stages, on each branch, were recorded. Branches were then sectioned, placed into 3.8 L freezer bags and stored at –20 ºC. Branch clippings were examined microscopically 4-6 wk later to obtain additional counts of predator net reproduction, and to assess predator feeding and impact on *A. tsugae*.

Predator Survival

Predator survival was assessed for individual females and sexual pairs of each species at the conclusion of each study. In conspecific groupings, survival was recorded for one female or sexual pair, selected at random, which was marked prior to the study. Survival was recorded as a binomial response: alive=1, dead=0, and was analyzed separately by predator species and season using a logistic regression (PROC LOGISTIC; SAS Institute, 2001). The model included predator species combination, year, and their interaction as independent categorical variables. The analyses only considered female survival since male predators were not present in 2003.

Predator Net Reproduction

Predator net reproduction, as well as feeding and impact on *A. tsugae*, were assessed by microscopic inspection of 200 cm of randomly selected clippings from each sectioned branch. Net reproduction was determined by counts of intact predator progeny, of all life stages. For conspecific treatments, the net reproduction per female predator was calculated. Eggs, larval and pupal lifestages were distinguished using morphological characteristics of *L. nigrinus* (Zilahi-Balogh et al. 2003a), *S. tsugae* (Sasaji and McClure 1997) and *H. axyridis* (Gordon 1985).

Predator Feeding and Impact on *A. tsugae*

Predator feeding was determined by counts of the number of sistens or progrediens ovisacs with evidence of predation; that is, when the adelgid ovisac was disrupted and the adult or progeny within showed evidence of being fed upon or killed by a predator. Predator feeding was distinguished from other causes of mortality by the presence of puncture wounds or damage to the exoskeleton that was consistent with previously observed predator attacks. Predation on *A. tsugae* in each combination was assessed as a total for all predators in the treatment, as feeding by individual species could not be distinguished. To assess predator impact during the spring, counts of the number of intact, developing progrediens remaining on branches at the completion of the study were made. Progrediens nymphs were judged to be alive and developing based on the presence of new woolly filaments. Counts of sexuparae were not included, since they naturally disperse from branches (McClure 1989, 1991b). Predator impact during the summer was determined by counts of the number of aestivating sistens present on the new growth of the branch sections.

Intra- and interspecific competition were assessed by comparing the responses of individual females or sexual pairs of each species to that of the average or combined responses of their respective conspecific grouping and all heterospecific groupings that contained the species being evaluated. Relative responses among these treatments were used to detect predator interference. For net reproduction, in the absence of significant competitive interference, we would expect similar responses from individual females or sexual pairs of each species and that of the heterospecific groupings containing that species. Similarly, the net reproduction per female, in conspecific groupings, should be similar to that of individual female or sexual pair treatments. In contrast, in the absence of significant competitive interference, we would expect total predator feeding and impact to be greater in conspecific and heterospecific groupings than in treatments with individual females or sexual pairs. Similarity among treatments for these two responses was used to indicate significant predator interference.

Statistical Analyses

Predator net reproduction, feeding and impact on *A. tsugae* were examined separately by season, and analyses of net reproduction were further separated by species. For each analysis, a general linear model (PROC GLM; SAS Institute, 2001) was used that included predator species combination, year, and their interaction as independent categorical variables. Results for these responses were $\log_{10}$ transformed to achieve normality and equality of variances, and analyzed using two-way analysis of variance (Zar 1998). All analysis of variance was followed by Tukey's honestly significant difference test to separate treatment means (Zar 1998), and results were evaluated for significance at $P \leq 0.05$.

Results

Predator Survival

Survival of predators was high overall and ranged between 61.1 and 83.3%. There was no interaction between the model variables for *L. nigrinus* (Wald χ^2 = 1.29; d.f. = 4; P = 0.86), *S. tsugae* (Wald χ^2 = 0.54; d.f. = 4; P = 0.96) and *H. axyridis* (Wald χ^2 = 1.23; d.f. = 4; P = 0.75) in the spring evaluation, or for *S. tsugae* (Wald χ^2 = 0.44; d.f. = 2; P = 0.84) and *H. axyridis* (Wald χ^2 = 0.47; d.f. = 2; P = 0.78) in the summer evaluation. Therefore, each factor was evaluated across other variable levels. For each species, in both spring and summer evaluations, there were no significant differences in survival that were related to predator species combination (Table 3.1). There were also no significant differences by year for *L. nigrinus* (Wald χ^2 = 0.51, d.f. = 1, P = 0.94), *S. tsugae* (Wald χ^2 = 0.09, d.f. = 1, P = 0.77), and *H. axyridis* (Wald χ^2 = 1.56, d.f. = 1, P = 0.21) in the spring, or *S. tsugae* (Wald χ^2 = 0.13, d.f. = 1, P = 0.72) and *H. axyridis* (Wald χ^2 = 0.28, d.f. = 1, P = 0.87) in the summer.

Table 3.1. Percent survival of adult female predators held in branch enclosures in the field after 6 wk during the spring and 4 wk during the summer. Predators were evaluated alone and combined with conspecific and heterospecific predators. *There were no significant differences by predator combination for any species during either season ($P \geq 0.05$).

Season (Prey Stage)	Predator Species[1]	Predator Species Combination[1]	*n*	Survival (%)	Wald $\chi 2$	**P*
Spring (Sistens)	*Ln*	Alone	19	78.9	0.05	0.97
		Ln+Ln	17	70.6		
		Ln+St	18	77.8		
		Ln+Ha	19	73.7		
		Ln+St+Ha	17	76.4		
	St	Alone	18	72.2	0.76	0.94
		St+St	19	73.7		
		St+Ln	18	78.9		
		St+Ha	16	75.0		
		St+Ln+Ha	17	70.6		
	Ha	Alone	19	68.4	0.21	0.96
		Ha+Ha	18	61.1		
		Ha+Ln	17	70.6		
		Ha+St	19	63.2		
		Ha+Ln+St	17	64.7		
Summer (Progrediens)	*St*	Alone	18	77.8	0.15	0.92
		St+St	19	73.7		
		St+Ha	19	78.9		
	Ha	Alone	17	82.4	0.28	0.87
		Ha+Ha	18	77.8		
		Ha+St	19	84.2		

[1] *Ln*: *Laricobius nigrinus*, *St*: *Sasajiscymnus* tsugae, *Ha*: Harmonia *axyridis*

Predator Net Reproduction

Analyses of predator reproduction showed no interaction between the variables for *L. nigrinus* (F = 1.66; d.f. = 4,85; P = 0.17), *S. tsugae* (F = 0.12; d.f. = 4,85; P = 0.97) or *H. axyridis* (F = 0.21; d.f. = 4,85; P = 0.93) in the spring, or for *S. tsugae* (F = 0.54; d.f. = 2,51; P = 0.59) or *H. axyridis* (F = 0.34; d.f. = 2,51; P = 0.72) in the summer. Therefore, each factor was evaluated across other variable levels. There were significant differences by predator species combination in the spring for *L. nigrinus* (F = 11.76; d.f. = 4,85; P < 0.0001) (Figure 3.2.A) and *H. axyridis* (F = 15.32, d.f. = 4,85, P < 0.0001) (Figure 3.2.C), but not *S. tsugae* (F = 0.87; d.f. = 4,85; P = 0.43) (Figure 3.2.B). The highest net reproduction during this period was by *L. nigrinus*, while *S. tsugae* and *H. axyridis* each had relatively low levels of net reproduction by comparison. Progeny of *L. nigrinus* were also more advanced developmentally than the coccinellid species, with greater larval (50.6%) and pre-pupal (36.2%) lifestages present in comparison to eggs (13.2%). For *S. tsugae*, progeny consisted of egg (70.8%) and larval lifestages (29.2%). Similarly, *H. axyridis* lifestages present were only egg (76.2%) and larvae (23.8%) during this evaluation. All species had lower net reproduction per predator in conspecific groupings; however, it was only significantly reduced for *L. nigrinus* and *H. axyridis* (Figure 3.2.A-C). Heterospecific groupings did not significantly affect net reproduction by these species. During the summer evaluation, net reproduction was similar, by predator species combination, for *S. tsugae* (F = 1.54; d.f. = 2,51; P = 0.21) (Figure 3.3.A), and significantly different for *H. axyridis* (F = 38.63; d.f. = 2,51; P < 0.0001) (Figure 3.3.B). For *S. tsugae*, progeny were again egg (69.4%) and larval (30.6%) lifestages, and this was consistent for *H. axyridis*, with only egg (66.5%) than larval (33.5%) lifestages present. Similar to the spring evaluation, *H. axyridis* had significantly reduced net reproduction in conspecific groupings, while heterospecific groupings showed no significant effect on either species. Net reproduction by year was similar for all predators in the spring: *L. nigrinus* (*F* = 3.16; d.f. = 1,85; *P* = 0.08), *S. tsugae* (*F* = 3.46; *d.f.* = 1,85; *P* = 0.07) and *H. axyridis* (*F* = 3.02; d.f. = 1,85; *P* = 0.09). The summer evaluation also showed no differences for *S. tsugae* (*F* = 1.89; d.f. = 1,51; *P* = 0.17) or *H. axyridis* (*F* = 3.60; d.f. = 1,51; *P* = 0.07).

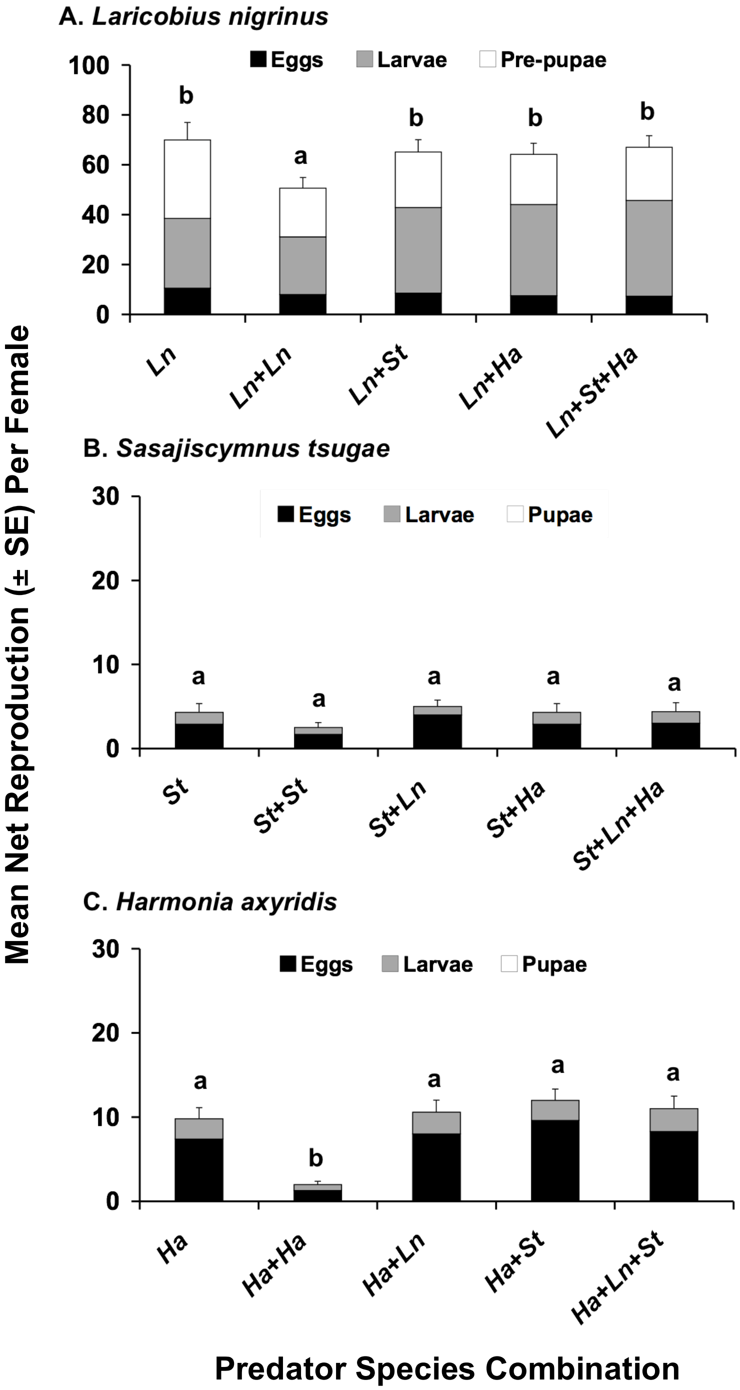

Figure 3.2. Mean net reproduction (±SE) per female predator, by species combination, for (A) *L. nigrinus* (*Ln*), (B) *S. tsugae* (*St*) and (C) *H. axyridis* (*Ha*) in the spring evaluation. Values represent the intact lifestages present at the conclusion of the study (n=20). Means with the same letter were not significantly different ($P \geq 0.05$).

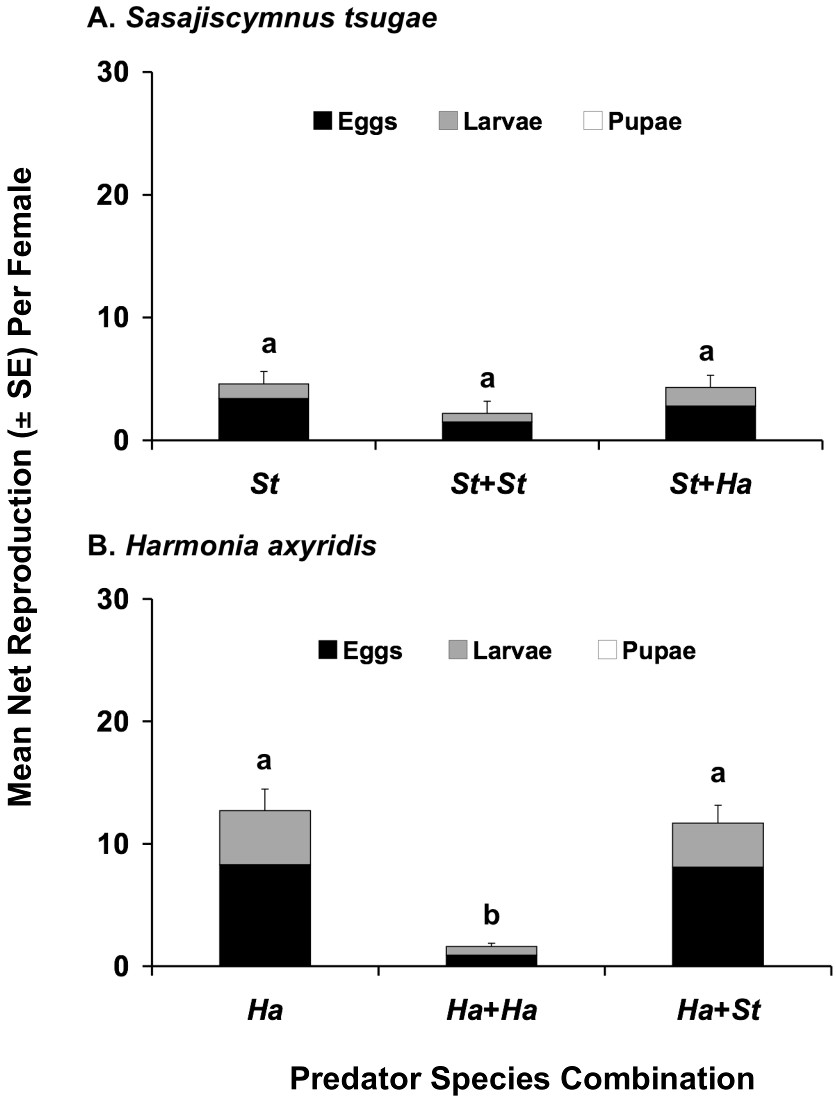

Figure 3.3. Mean net reproduction (±SE) per female predator, by species combination, for (A) *S. tsugae* (*St*) and (B) *H. axyridis* (*Ha*) in the summer evaluation Values represent the intact lifestages present at the conclusion of the study (*n*=20). Means with the same letter were not significantly different ($P \geq 0.05$).

Predator Feeding on *A. tsugae*

For predator feeding on *A. tsugae*, there were no interactions between model variables during the spring (F = 1.66; d.f. = 10,187; P = 0.10) or summer (F = 1.74; d.f. = 5,102; P = 0.13), so each factor was evaluated across other variable levels. Feeding was significantly different by predator species combination during the spring (F = 430.40; d.f. = 10,187; $P \leq$ 0.0001) (Figure 3.3.A) and summer (F = 172.41; d.f. = 5,102; $P \leq$ 0.0001) (Figure 3.3.B). In the spring, individual species comparisons showed that *L. nigrinus* and *H. axyridis* fed on significantly more sistens than *S. tsugae*, and in summer, *H. axyridis* fed on significantly more progrediens than *S. tsugae*. Comparisons of individual predator species, with their respective conspecific and heterospecific groupings revealed that total feeding was significantly higher for all species when placed in predator groupings. During the spring, the greatest amount of feeding occurred in the three-species treatment, while the conspecific grouping of *H. axyridis* had the highest amount of feeding during summer. All predator treatments showed significantly more feeding than control branches during each season. Analyses by year indicated that there was significantly more feeding in 2004 during the spring (F = 38.35; d.f. = 1,187; $P \leq$ 0.0001) and summer (F = 90.05; d.f. = 1,102; $P \leq$ 0.0001).

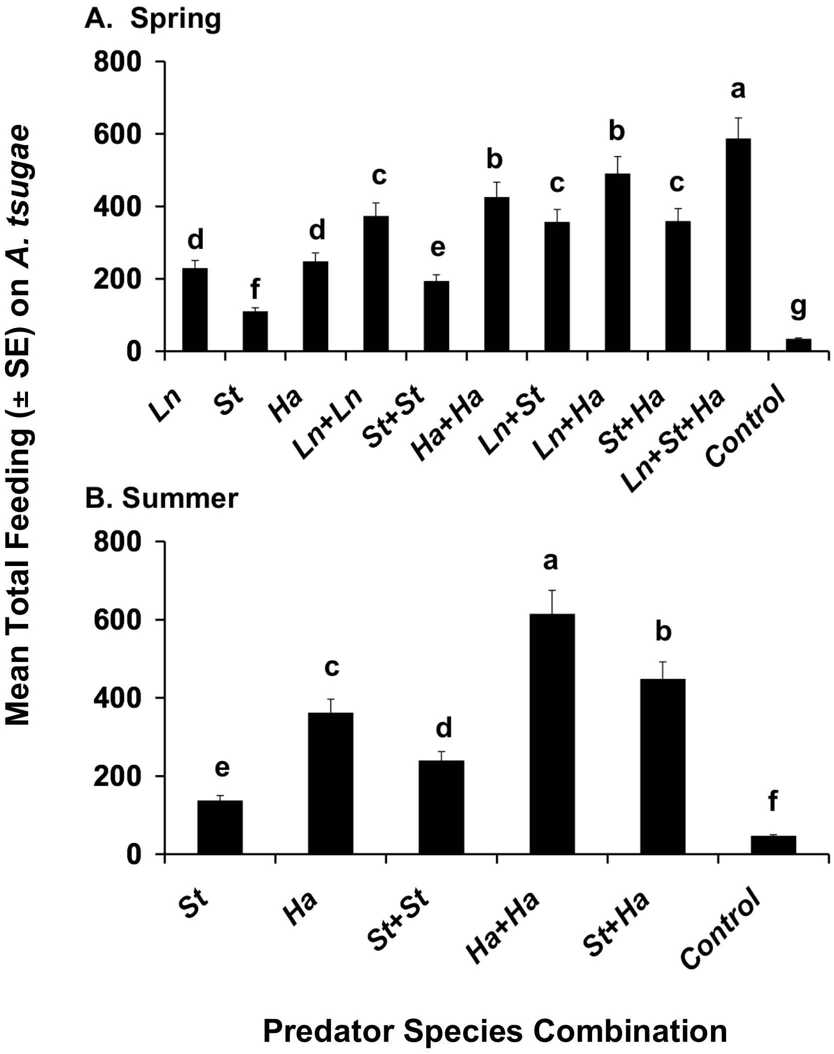

Figure 3.4. Mean total feeding (±SE) on *A. tsugae* (A) sistens in spring and (B) progrediens in summer by *L. nigrinus* (*Ln*), *S. tsugae* (*St*), and *H. axyridis* (*Ha*). Feeding corresponded to the number of *A. tsugae* adults and/or ovisacs that showed evidence of disruption and/or predation. Each species was evaluated alone and in combination with one conspecific, one heterospecific and with all species together (n=20). Means with the same letter were not significantly different ($P \geq 0.05$).

Predator Impact on *A. tsugae*

Analyses of predator impact on *A. tsugae* again showed no interaction between model variables in either spring (*F* = 1.17; d.f. = 10,187; *P* = 0.32) or summer (*F* = 1.23; d.f. = 5,102; *P* = 0.30), so each factor was evaluated across other variable levels. There were significant differences by predator species combination in the spring (*F* = 186.29; d.f. = 10,187; $P \leq 0.0001$) (Figure 3.4.A) and summer (*F* = 30.17; d.f. = 5,102; $P \leq 0.0001$) (Figure 3.4.B). Comparisons among the three species showed that *L. nigrinus* had significantly greater impact than *S. tsugae* or *H. axyridis* during the spring, and *H. axyridis* had greater impact than *S. tsugae* during the summer. Comparisons among spring predator treatments revealed that *L. nigrinus* and *H. axyridis* had significantly greater impact with conspecifics, in heterospecific groupings with one another and with all three species. For *S. tsugae*, there was significantly greater impact in all heterospecific groupings, while conspecifics did not improve their impact. In the summer evaluation, results for *S. tsugae* again showed significantly improved impact in heterospecific groupings, while conspecifics did not improve results. For *H. axyridis*, impact was significantly improved by conspecifics, but not by the heterospecific combination. All predator treatments showed significantly greater impact than control branches during each season. Predator impact by year was greater in 2004 during the spring (*F* = 84.95; d.f. = 1,187; $P = \leq 0.0001$) and summer (*F* = 11.42; d.f. = 1,102; *P* = 0.001).

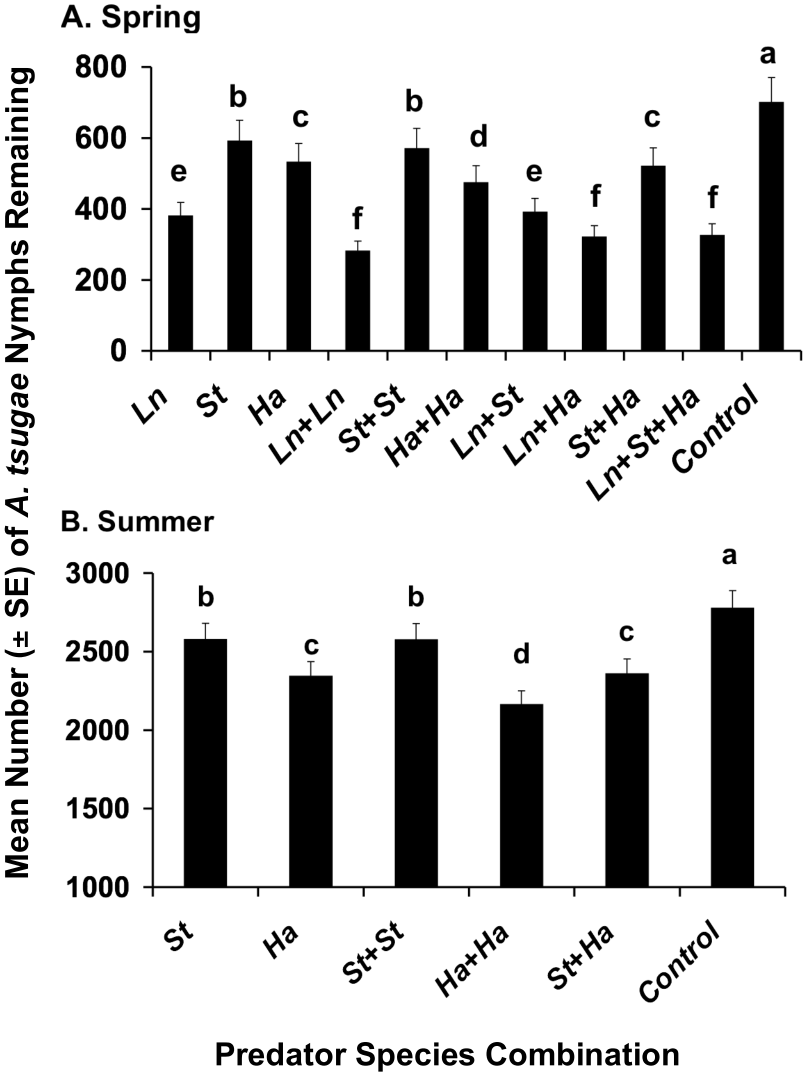

Figure 3.5. Mean number (±SE) of *A. tsugae* (A) progrediens nymphs remaining at the conclusion of the spring evaluation and (B) sistens nymphs remaining at the conclusion of the summer evaluation. Each species was evaluated alone and in combinations with conspecific, one heterospecific and with all species together (*n*=20). Means with the same letter were not significantly different ($P \geq 0.05$).

Discussion

Cannibalism and interspecific predation by adult females was not observed to a significant degree in these studies. Although *H. axyridis* has the potential to be a voracious intraguild competitor (rev. in Koch 2003), these results indicated that predator survival was not impacted by direct competition. This was likely due to the high host densities of the branch enclosures and the reduced activity of the *H. axyridis* at low field temperatures. Overall, these species had high survival rates, providing further evidence that these predators are well adapted to surviving in hemlock stands in the southeastern United States.

Although *L. nigrinus* had the highest net reproduction in the spring, previous studies indicate that *S. tsugae* and *H. axyridis* have much higher lifetime mean fecundity in the laboratory (Cheah and McClure 1998, Stathas et al. 2001). However, higher temperatures are required for these coccinellid species than occurred in the spring evaluation. The developmental threshold for *L. nigrinus* is lower than that of *S. tsugae* or *H. axyridis* by 5.8 and 7.5ºC, respectively (Cheah and McClure 1998, LaMana and Miller 1998, Zilahi-Balogh et al. 2003b). Because reproduction by all species is influenced by temperature (Cheah and McClure 1998, Stathas *et al*. 2001, Zilahi-Balogh *et al*. 2002), the lower threshold of *L. nigrinus* may have allowed increased reproduction at low field temperatures. The progeny of *L. nigrinus* were also more advanced, developmentally, than the other species, indicating earlier reproductive activity. Temperatures and photoperiods during the summer evaluation were similar to optimal rearing conditions for the coccinellids; however, reproduction may still have been limited due to prey quality (Palmer and Sheppard 2002), host tree health (Sheppard and Palmer 2004) or nutrient limitation. In addition, branch cages limited predator dispersal and choice of potentially more favorable microhabitats within the stand.

Reproductive interference by conspecifics was evident to varying degrees. Negative effects were most apparent for *H. axyridis* and *L. nigrinus*, while *S. tsugae* displayed a lesser degree of interference. Previous studies of *H. axyridis* indicate that indirect reproductive interference occurs by passive substrate marking using fecal cues (Agarwala et al. 2003) and oviposition-deterring pheromones (Yasuda et al. 2000). Direct interference by egg cannibalism also occurs (Osawa 1992), and provides increased nutrition and growth when prey is of low quantity or quality (Wagner et al. 1999, Yasuda and Ohnuma 1999, Snyder et al. 2000, Michaud and Grant 2004). In these studies, both direct and indirect competition occurred. Microscopic examination of branch clippings showed remnant chorions of *H. axyridis* eggs due to cannibalism. However, it appeared that oviposition did not occur initially in most cases, and that reproductive interference for *H. axyridis* was by indirect means. The chemical ecology exhibited by this species has not been investigated for the specialist predators, but similar mechanisms may be used. In contrast, heterospecific reproductive interference did not occur to a significant degree. Although predation of heterospecific progeny by *H. axyridis* is well documented (Yasuda and Shinya 1997, Burgio et al. 2002), it was likely limited in these studies because of high host densities and low temperatures. In contrast, chemical deterrents in eggs (Pasteels et al. 1973) and defensive spines of larvae (Dixon 2000) may have prevented interspecific predation of *H. axyridis* life stages. Net reproduction was similar in both years of the study for spring and summer, indicating that environmental conditions may be the limiting factor for the coccinellid species, and not insufficient mating of females.

Feeding by predators was also greatly influenced by ambient temperatures. In the spring, the larger and more voracious *H. axyridis*, and smaller *L. nigrinus*, had similar amounts of feeding due in part to decreased activity by *H. axyridis* and higher reproduction by *L. nigrinus* at the low environmental temperatures (Lamb et al. 2005a). In contrast, predator feeding in summer was positively associated with body size, with the larger *H. axyridis* feeding more than *S. tsugae*. Feeding by the coccinellid species was greater in summer than in spring, supporting studies that show these predators to be more active during summer (Lamana and Miller 1998, Cheah and McClure 2000). The incorporation of additional conspecific and heterospecific predators significantly increased the total amount of feeding for each species. It was not possible to determine if per predator feeding averages increased (synergistic effects), but it does indicate that predator interference did not occur to such the degree that significant negative effects occurred. Predator feeding was greater in 2004, and this may be attributable to the inclusion of male predators or more favorable environmental conditions. Limited feeding on *A. tsugae* on un-caged control branches indicates that native species are not affecting *A. tsugae* densities in these hemlock stands.

Predator impact on *A. tsugae* population densities varied substantially between seasons. In spring *L. nigrinus* had the greatest impact, and this is partly attributable to adult feeding, but more importantly, resulted from high net reproduction. The number of progeny produced in each trial consumed a large number of eggs in sistens ovisacs, thus limiting the growth of the progrediens generation. During summer, *H. axyridis* had the greatest impact on the progrediens and the number of aestivating sistens remaining, although the reductions in this season were much lower by comparison. The coccinellid predators concentrated feeding primarily on *A. tsugae* adults in each season that had already produced eggs. This factor coupled with reduced reproduction led to a lower degree of impact on the following generation of *A. tsugae*. Due to the amount of reproduction and feeding by *L. nigrinus* and *H.*

axyridis during these trials, predator combinations involving these species had greater impact than *S. tsugae*, which appeared to display limited activity, and appeared to be more sensitive to experimental conditions. In each season, predator treatments reduced the number of developing or aestivating *A. tsugae* nymphs relative to caged and un-caged controls; however, it is unknown if these reductions are sufficient to reduce future adelgid densities below injurious levels. Predator impact in 2004 was greater during both seasons, and this may be attributable to increased activity by predators under the more favorable environmental conditions. Overall, it appears that it is the production of predator progeny and their subsequent activity that has the greatest impact on future *A. tsugae* generations.

Competitive interactions among predators may directly or indirectly affect predator diversity and biological control (Rosenheim et al. 1993, Polis et al. 1989, Lucas et al. 1998). Initial field studies of these three predators of *A. tsugae* indicate that conspecific interference may negatively affect these species, while heterospecific interference was limited. These results do not provide definitive conclusions as to the level of competition that may occur among these species under different ecological conditions or longer durations. The experimental design has several constraints, as branch enclosure studies do not reflect the potential for predator immigration and emigration in response to changing environmental conditions or prey abundance. In addition, the level of competition among predators may have been constrained by the reduced activity of the coccinellid species under low temperature field conditions. However, this study accurately represents the conditions that may occur during the seasonal overlap of these species, and the results suggest that significant interspecific competition does not occur.

Chapter 4

Video Analyses of Predator Behavior and Daily Activity Patterns

Introduction

The hemlock woolly adelgid, *Adelges tsugae* Annand is a major pest of eastern hemlock, *Tsuga canadensis* L. Carriere, and Carolina hemlock, *T. caroliniana* Engelmann, in the eastern United States. Two specialist predators, *Laricobius nigrinus* Fender and *Sasajiscymnus tsugae* Sasaji and McClure are currently being released as part of the biological control program for *A. tsugae*. In addition, *Harmonia axyridis* Pallas, an established, exotic generalist predator, is now commonly found with *A. tsugae* (Wallace and Hain 2000). Direct or indirect competition among species using the same resource at the same time may lead to reductions in the effectiveness of the predators (Rosenheim et al. 1993). In this study, individual predator behaviors and daily activity patterns were examined in the laboratory using video recording methods. Interactive behavior and ecology of these species has not been studied, and it is unknown if avoidance behaviors mediate interactions among these species. Therefore, this study was undertaken to evaluate the searching, feeding, resting, and oviposition behavior of all three predator species with *A. tsugae* and to document their daily temporal and spatial activity patterns. The effect of additional conspecific and heterospecific predators on these responses was also examined.

Methods and Materials

Insect Cultures

Laricobius nigrinus adults were obtained from a colony reared at Virginia Polytechnic Institute and State University (Blacksburg, Virginia). *Sasajiscymnus tsugae* adults were obtained from colonies at Clemson University (Clemson, South Carolina) and the University of Tennessee (Knoxville, Tennessee). *Harmonia axyridis* adults were collected locally in southwestern Virginia (Jefferson National Forest, Virginia). All predators were reared under their normal developmental conditions at 10°C, LD 12:12 h, 75% RH for *L. nigrinus* (Lamb et al. 2005b), and at 25°C, LD 16:8 h, 45% RH for both coccinellid predators (Palmer and Sheppard 2002, Matsuka and Niijima 1985). *Sasajiscymnus tsugae* species were stored at 20°C, LD 16:8 h, 45% RH before shipment to our laboratory (Palmer and Sheppard 2002). Upon receiving the predators, conditions were gradually stepped down from 20 to 12°C and from LD 16:8 to LD 12:12 over a 2-3 wk period to precondition predators to the conditions used in the spring evaluation. At the conclusion of the spring trials, conditions were gradually increased from 12 to 22°C and from LD 12:12 to LD 16:8 over the same time interval to precondition predators for the summer evaluation.

All species were held in 2.2 L plastic containers lined with moistened filter paper and ventilated with fine polyester mesh (Sefar). Each container held 15 adults, in a sex ratio of 2F:1M, and 5-7 *T. canadensis* branch clippings heavily infested with *A. tsugae*. Containers were maintained in an environmental chamber (Percival-Scientific). Adult predators were transferred from holding containers to assays 24 h prior to recording. All predator females were mature, of approximately the same age (12-24 wk), and were selected randomly from pre-conditioning containers. Morphological characters were used to separate the sex of *H. axyridis* (Gordon 1985) and *S. tsugae* (Sasaji and McClure 1997), while monitoring oviposition was used for *L. nigrinus*.

Experimental Design of Video Studies

Predator behavior and daily activity patterns of adult females of each species were documented in the laboratory using intermittent digital video recording. Predator arenas consisted of 5 x 2.5 cm Petri dishes (Fisher-Scientific) lined with moistened filter paper. Experiments were conducted in an environmentally controlled room using two evaluation periods based on temperature and RH averages obtained by data loggers (Onset Computers) placed in hemlock stands in southwestern Virginia. The evaluations were termed 1) spring: 12±2˚C, LD 12:12 h, 50-75% RH and 2) summer: 22±2˚C, LD 16:8 h and 65-85% RH. These conditions cover the approximate duration when adult *A. tsugae* sistens and progrediens with ovisacs are present (McClure 1989). Each assay contained two 5 cm hemlock clippings, placed in parallel alignment, which were infested with 10 *A. tsugae* sistens (spring) or progrediens (summer) adults with ovisacs. Each predator species was evaluated alone, and combined with one additional conspecific or heterospecific predator in all possible combinations. All species were included in the spring evaluation; however, *L. nigrinus* was excluded from the summer evaluation due to its natural aestivation period (Zilahi-Balogh et al. 2003b).

Video capture was done using a one-chip MiniDV camcorder (Panasonic PV-GS35) that was mounted on a camera stand 5-10 cm directly above the arena (Figure 4.1). The camcorder had a 30X optical lens and 1000X digital zoom with digital image stabilization.

Automatic settings were generally used to control camcorder exposure, gain, focus and white-balance levels; however, manual adjustments were made as necessary to improve overall video quality. A FireWire video output connection was used to join the camcorder to an iMac G4 computer (Apple Computer) (Figure 4.1). EvoCam 3.5 software (Evological) was used to visualize the arena, control video-capture and compress video segments prior to storage on the computer harddrive. One-minute video recordings were captured every 15 min over 24 h, resulting in a total of 96 observations per day. Each treatment was prepared in duplicate, with one assay recorded during the day and one at night. Video segments were compressed to MPEG format and reviewed using QuickTime 7.0 software (Apple Computer). Two light sources with white translucent diffusing filters were positioned 0.5-1 m on each side of the arena to illuminate the area adequately for filming. Day recordings used ambient light supplemented by 20W soft-white light (Phillips), while night recordings used 20W red light (Phillips). Pilot studies were completed to determine optimal assay conditions for filming and to assure that predator behavior was not significantly altered in response to experimental conditions. A data logger (Onset Computer) was placed next to the assay to monitor room environmental conditions. Six replications of each treatment were completed, three during the day and three at night.

Figure 4.1. Experimental arena for the video studies. Each assay consisted of a 5 x 2.5 cm polystyrene Petri dish lined with moistened filter paper, and held 10 cm of branch clippings of eastern hemlock, *T. canadensis*, infested with *A. tsugae*. A digital video camera, mounted on a camera stand 5-10 cm above the assay was used for video capture. All intermittent video capture was controlled using EvoCam 3.5 software and video segments were visualized and stored on an Apple iMac G4 computer.

Predator Behavior and Daily Activity

All video recordings were reviewed and scored as to the behavior category exhibited and relative location of each predator. Predator behavior was classified into five general categories: 1) Extensive searching, 2) Intensive searching, 3) Feeding, 4) Oviposition or 5) Resting. The duration of each behavior was consistenty greater than the 1 min recording interval, so each video segment was assigned to a single behavioral category. Searching behavior was divided into extensive search, distinguished by rapid movement over a large area of the assay or branch surface (Video 4.1), and intensive search, distinguished by slower movements over a small area of the branch (Video 4.2). Predator placement of the head or mouthparts within *A. tsugae* ovisacs for the duration of the video segment was used to designate feeding behavior (Video 4.3). This was often accompanied by visual evidence of disrupted woolly filaments and the presence of adelgid hemolymph on the ovisac surface. For *L. nigrinus*, close inspection of *A. tsugae* ovisacs followed by insertion of the functional ovipositor was used to designate oviposition behavior (Video 4.4, Video 4.5); however, *S. tsugae* and *H. axyridis* oviposition could often not be directly observed due to experimental limitations. Oviposition by these species would often occur with the female directly over branch or needle substrates, which blocked the field of view and made it difficult to distinguish from resting behavior, as only a single, overhead view of the arena was available. Therefore, oviposition was determined indirectly by microscopic examination of branch clippings at the conclusion of each trial. For *S. tsugae*, which lays eggs singly, each egg found was designated as a single oviposition event for that time period. For *H. axyridis*, which lays eggs together at a single location, each egg cluster found was scored as an oviposition event. Predator eggs were distinguished using morphological characteristics of *S. tsugae* (Sasaji and McClure 1997) and *H. axyridis* (Gordon 1985). Lastly, resting behavior was designated as predators maintaining a stationary position on the branch or assay surface for the duration of the video segment (Video 4.5).

Behavior descriptions for each species were based on a review of 18 video segments of each behavior taken from single-predator assays. This included 3 video segments of each behavior from each of the experimental replicates. Predator daily activity patterns were documented by determining the percent of time each species allocated to each behavior category during each time period (day/night) over the 24 hr evaluation period. This was calculated by dividing the number of observations of each behavior category by the total number of scored observations in each time period. The total included direct visual assessments of video segments showing predator searching, feeding, resting or oviposition behaviors, as well as indirect assessments of predator oviposition for the coccinellid species. Video recordings in which predator behavior could not readily be distinguished or consisted of multiple behaviors were excluded.

Predator Effects on Temporal and Spatial Patterns

In multiple-predator assays, behavior was scored for one marked predator, chosen at random, in conspecific pairings and for each species in heterospecific pairings. The impacts of additional conspecific and heterospecific predators on daily activity patterns were assessed by comparison of the results from single- and multiple-predator trials. In the absence of significant competitive interference, we would expect similarity among the percent of time allocated to each behavior among the treatments. A significant shift in time allocation was used to indicate predator interference. For spatial analyses of predator pairs, the approximate separation (0-5 cm) between predators was assessed at the start of each recording, and distances were averaged for each treatment. Separation distances were measured from the center of the body of each predator to account for size differences among these species.

Statistical Analyses

Predator temporal and spatial patterns were examined separately for each evaluation period using general linear models (PROC GLM; SAS Institute 2001). Single-predator analyses were examined separately by species and the model included predator behavior category and time (day and night) as independent categorical variables. For multiple-predator temporal analyses, the model included predator combination and behavior category. For multiple-predator spatial analyses, the model included predator combination and time. Treatment means were analyzed using two-way analysis of variance followed by Tukey's honestly significant difference test to separate treatment means (Zar 1998). All percent data were arcsin square root transformed to achieve normality and equality of variances, and results were evaluated for significance at $P \leq 0.05$.

Results

Predator Behavior

Predator behavior varied qualitatively for each species. Extensive searching behavior was similar in form for all species. It occurred primarily on the upper and lower assay surfaces and consisted of successive rapid movements covering a large area. The behavior was often followed by re-location onto branch surfaces, and a rapid shift to intensive searching behavior occurred. In other instances, the behavior would occur for longer periods of time, and may represent an attempt at dispersal from the arena. For *L. nigrinus*, intensive searching involved inspecting the majority of *A. tsugae* in the assay using a series of short linear movements from one prey location to another. During inspection, there was repeated tapping of the antennae and abdomen on the surface of the sistens ovisac. After surveying numerous prey items, adult females would move to one ovisac and often shift to feeding or oviposition behavior. Intensive searching by *S. tsugae* consisted of a series of systematic movements along the needle and branch surfaces. Often, one side of the branch clipping would be surveyed at a time by horizontal movements along each side of several needles in succession. This usually resulted in direct contact with *A. tsugae*, after which this search pattern would often cease and an apparent closer inspection of prey would occur. For *H. axyridis*, intensive searching appeared to be less systematic than the specialists. Females would often move from resting locations directly to the prey in closest proximity. Selection was rapid and feeding often began immediately.

Feeding by adult female predators was similar in form for these species and was focused on adult *A. tsugae* sistens or progrediens in each trial. The mandibles were used to tear the ovisac filaments to obtain access to the adult, and feeding would occur on adelgid honeydew and hemolymph, often resulting in a punctured and collapsed *A. tsugae* exoskeleton. Feeding by *L. nigrinus* occurred more often and for shorter durations than *S. tsugae* or *H. axyridis*. Female *L. nigrinus* would often attempt to access the adult from the side or bottom of the ovisac, resulting in lower levels of disruption to the woolly filaments than the coccinellid predators, which often accessed *A. tsugae* directly through the top of the ovisac, resulting in substantial disruption of the filaments.

In contrast, predator oviposition behavior was widely distinct in form and location. For *L. nigrinus*, there was extensive tapping of the antennae and abdomen on sistens ovisacs, and often several insertions of the ovipositor would occur prior to egg deposition. The ovipositor would also be driven deep into the ovisac by lateral movements of the legs and abdomen. Oviposition for this species was diffuse overall, but 2-3 eggs would sometimes be placed within the same ovisac. Indirect assessments showed that oviposition by *S. tsugae* and *H. axyridis* was low overall. For *S. tsugae*, oviposition was diffuse, with eggs placed singly at concealed locations on the bud scales and in bark crevices. In contrast, oviposition by *H. axyridis* occurred in multiples of 5-15 eggs placed primarily on needle surfaces.

Resting behavior among these species also varied by location. For *L. nigrinus* and *S. tsugae*, resting occurred primarily at concealed locations on the branch surface in close proximity to *A. tsugae*. Predators were often located at the junction of the stem and a branchlet or between two needles at the base of a branchlet. Resting by *H. axyridis* occurred at exposed locations on branch and arena surfaces at greater distances from *A. tsugae*.

Predator Daily Activity

In the single-predator trials, there was no interaction between the variables for *L. nigrinus* ($F = 2.38$; df = 4,20; $P = 0.09$), *S. tsugae* ($F = 0.54$; df = 4,20; $P = 0.71$) and *H. axyridis* ($F = 1.91$; df = 4,20; $P = 0.15$) in the spring, or for *S. tsugae* ($F = 1.04$; df = 4,20; $P = 0.41$) and *H. axyridis* ($F = 2.61$; df = 4,20; $P = 0.07$) during the summer. Therefore, each factor was evaluated across other variable levels. During the spring, there were significant differences by behavior category for *L. nigrinus* ($F = 35.61$; df = 4,20; $P \leq 0.0001$) (Figure 4.2.A), *S. tsugae* ($F = 25.16$; df = 4,20; $P \leq 0.0001$) (Figure 4.2.B) and *H. axyridis* ($F = 41.52$; df = 4,20; $P \leq 0.0001$) (Figure 4.2.C). For *L. nigrinus*, intensive searching, resting and feeding behaviors were most common, while oviposition and extensive searching occurred less frequently. For *S. tsugae* and *H. axyridis*, resting behavior occurred most frequently, followed by searching, feeding and oviposition, which were low overall. During the summer evaluation, significant differences also occurred by behavior category for *S. tsugae* ($F = 103.22$; df = 4,20; $P \leq 0.01$) (Figure 4.3.A) and *H. axyridis* ($F = 43.41$; df = 4,20; $P \leq 0.01$) (Figure 4.3.B). The overall pattern was similar to the spring evaluation; however, percent resting was decreased, while percent searching, feeding and oviposition increased for both species. In the spring evaluation, there were no significant differences by time for *L. nigrinus* (*F* = 0.09; df = 1,20; *P* = 0.77), *S. tsugae* (*F* = 0.01; df = 1,20; *P* = 0.91) and *H. axyridis* (*F* = 1.61; df = 1,20; *P* = 0.22). Predator behavior by time was also similar in the summer evaluation for *S. tsugae* (*F* = 0.51; df = 1,20; *P* = 0.52) and *H. axyridis* (*F* = 0.25; df = 1,20; *P* = 0.62).

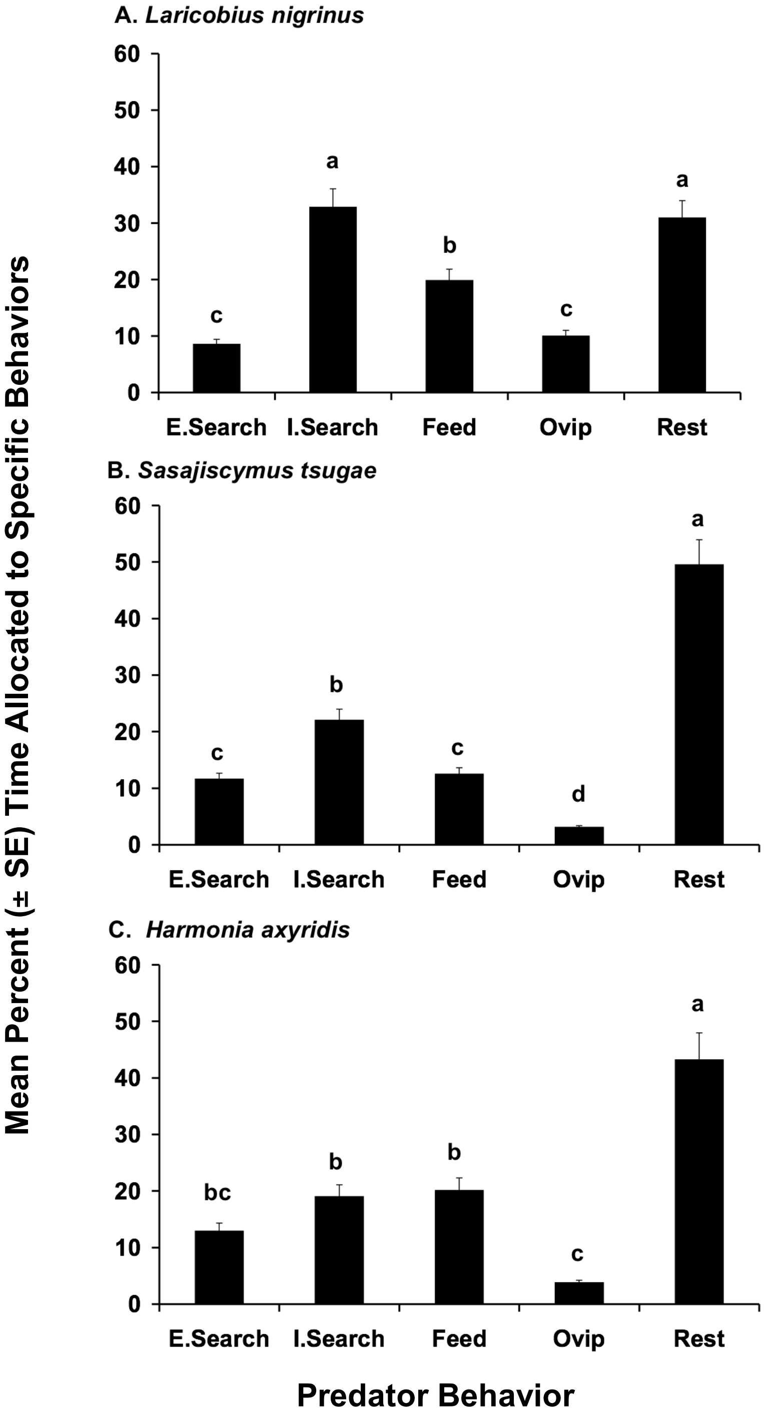

Figure 4.2. Mean percent (± SE) of time adult female (A) *L. nigrinus*, (B) *S. tsugae* and (C) *H. axyridis* allocated to five behaviors (Extensive Searching, Intensive Searching, Feeding, Resting and Oviposition) during the spring evaluation in single-predator trials (n=6). One-minute video segments were captured every 15 min over 24 h (≈ 96 observations). Means with the same letter were not significantly different (P ≥ 0.05).

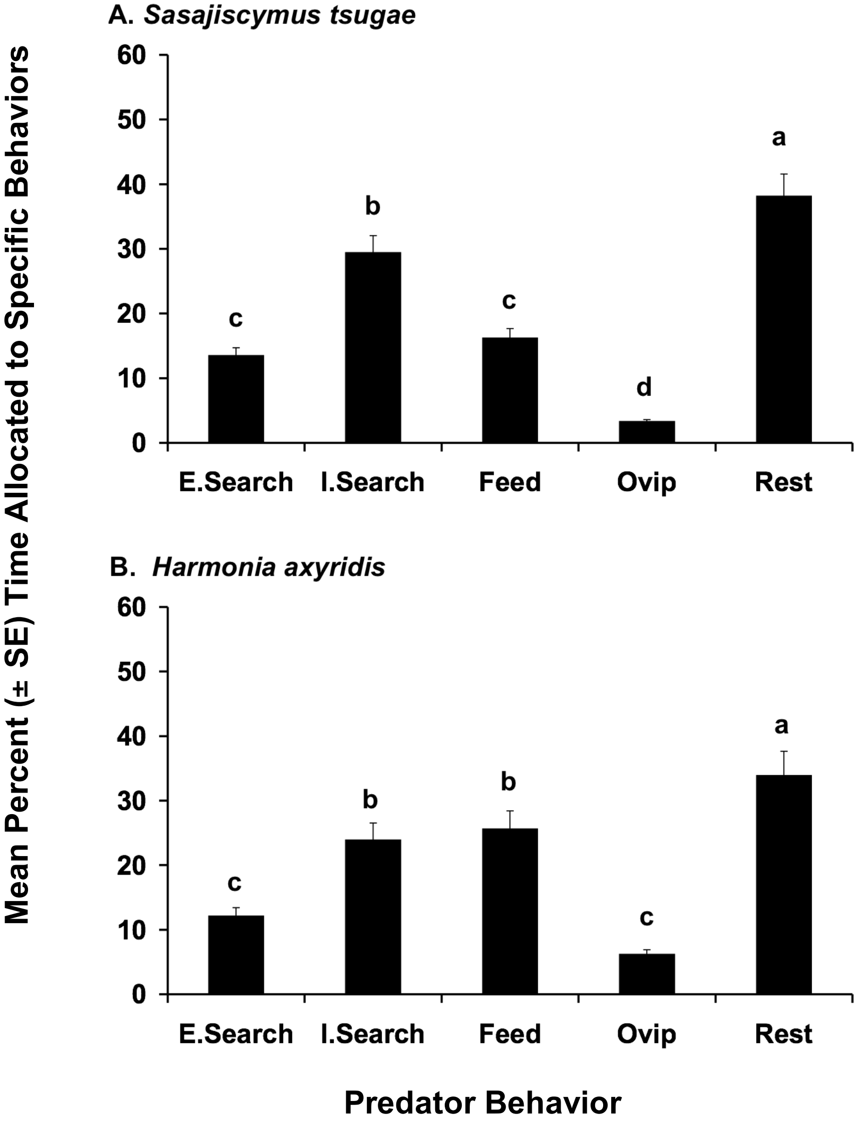

Figure 4.3. Mean percent (± SE) of time adult female (A) *S. tsugae* and (B) *H. axyridis* allocated to five behaviors (Extensive Searching, Intensive Searching, Feeding, Resting and Oviposition) during the summer evaluation in single-predator trials (n=6). One-minute video segments were captured every 15 min over 24 h (≈ 96 observations). Means with the same letter were not significantly different (P ≥ 0.05).

Predator Effects on Temporal Patterns

For temporal activity comparisons in the multiple-predator trials, there were significant interactions between the variables for *L. nigrinus* (*F* = 3.52; df = 12,80; *P* = 0.003) in the spring and for *H. axyridis* in the spring (*F* = 2.04; df = 12,80; *P* = 0.03) and summer (*F* = 11.32; df = 12,80; $P \leq 0.0001$). Therefore, each behavior was analyzed separately for these species. For *S. tsugae* there were no significant interactions among these variables in spring (*F* = 1.11; df = 12,80; *P* = 0.36) or summer (*F* = 1.73; df = 12,80; *P* = 0.11), so each factor was evaluated across the other variable levels. In the spring evaluation, *L. nigrinus* showed significant differences by predator combination for extensive searching (*F* = 3.42; df = 3,16; *P* = 0.04), intensive searching (*F* = 3.24; df = 3,16; *P* = 0.05) and resting (*F* = 3.86; df = 3,16; *P* = 0.02), while feeding (*F* = 2.22; df = 3,16; *P* = 0.12) and oviposition (*F* = 2.12; df = 3,16; *P* = 0.13) were similar (Figure 4.4.A). It was the conspecific assay that varied in each case, leading to greater searching and decreased resting. In contrast, *S. tsugae* showed similar responses for each behavior by predator combination (*F* = 0.13; df = 4,80; *P* = 0.84) (Figure 4.4.B). For *H. axyridis*, there were significant differences by predator combination for extensive searching (*F* = 5.02; df = 3,16; $P \leq 0.0001$), oviposition (*F* = 2.65; df = 3,16; *P* = 0.031) and resting (*F* = 2.82; df = 3,16; *P* = 0.04), while intensive searching (*F* = 0.86; df = 3,16; *P* = 0.48) and feeding (*F* = 1.51; df = 3,16; *P* = 0.25) were similar (Figure 4.4.C). It was again the conspecific treatment that varied in each case, leading to increased extensive searching and reduced resting and oviposition. During the summer evaluation, *S. tsugae* was again similar by predator combination for each behavior category (*F* = 0.80; df = 4,80; *P* = 0.56) (Figure 4.5.A). In contrast, *H. axyridis* showed significant differences in extensive searching (F = 48.5; df = 2,12; P, P $\leq$ 0.0001), feeding (F = 14.69; df = 2,12; $\leq$ 0.0001), oviposition (F = 3.53; df = 2,12; P = 0.024) and resting (F= 12.33; df = 2,12; $P \leq$ 0.0001), but not intensive searching (F = 0.86; df = 2,12; P = 0.48) (Figure 4.5.B). During this evaluation, conspecifics increased extensive searching and reduced feeding, oviposition and resting behaviors.

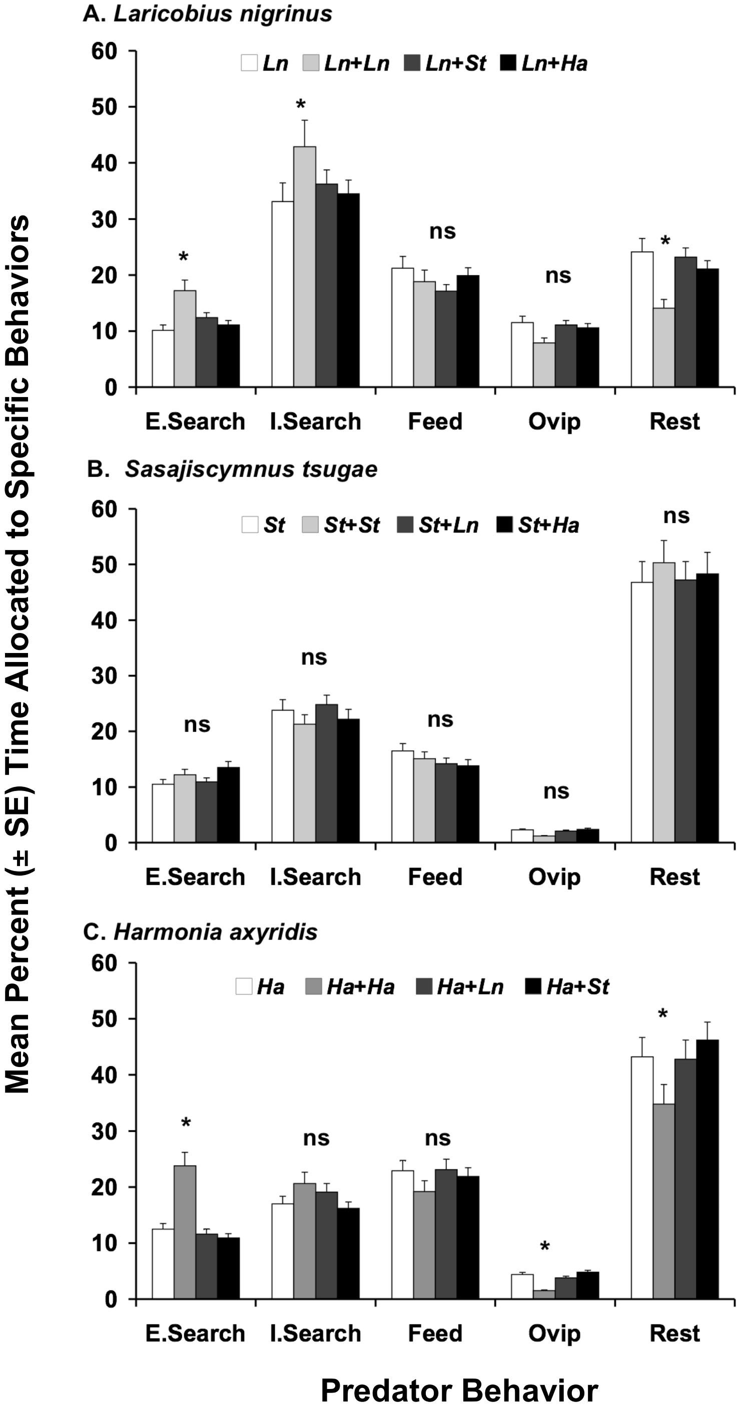

Figure 4.4. Mean percent (± SE) of time adult female (A) *L. nigrinus* (*Ln*), (B) *S. tsugae* (*St*) and (C) *H. axyridis* (*Ha*) allocated to five behaviors (Extensive Searching, Intensive Searching, Feeding, Resting and Oviposition) during the spring evaluation (n=6). Each species was evaluated alone and in combination with one conspecific or heterospecific predator. One-minute video segments were captured every 15 min over 24 h (≈ 96 observations). Analyses were done separately for each behavior: *Mean was significantly different from the other treatments ($P \leq 0.05$), ns = no significant difference among means.

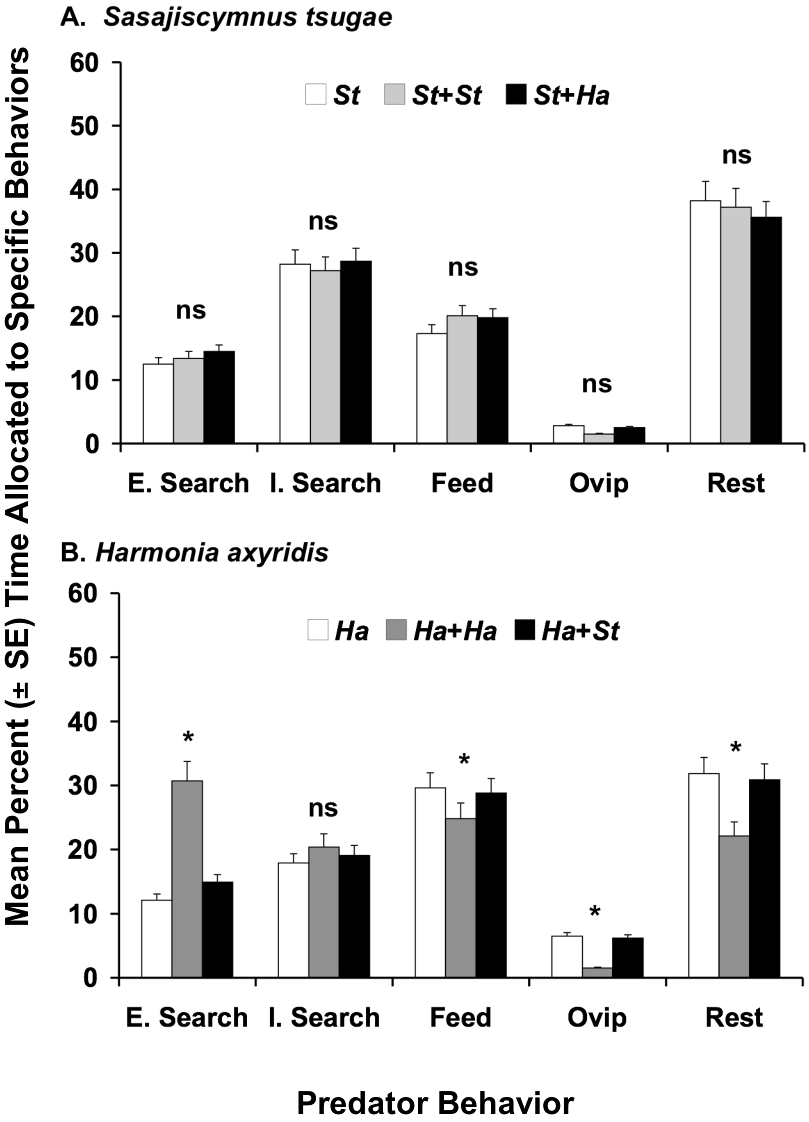

Figure 4.5. Mean percent (Mean ± SE) of time adult female (A) *S. tsugae* (*St*) and (B) *H. axyridis* (*Ha*) allocated to five behavior (Extensive Searching, Intensive Searching, Feeding, Resting and Oviposition) during the summer evaluation (n=6). Each species was evaluated alone and in combination with one conspecific or heterospecific predator. One-minute video segments were captured every 15 min over 24 h (≈ 96 observations). Analyses were done separately for each behavior: *Mean was significantly different from other treatments for that behavior ($P \leq 0.05$), ns = no significant difference among means.

Predator Effects on Spatial Patterns

For spatial activity comparisons in the multiple-predator trials, there were no significant interactions between the variables during spring ($F = 1.84$; df = 5,24; $P = 0.14$) or summer ($F = 0.08$; df = 2,12; $P = 0.92$), so factors were evaluated across other variable levels. There were significant differences by predator combination during both spring ($F = 14.07$; df = 5,24; $P \leq 0.0001$) (Figure 4.6.A) and summer ($F = 56.63$; df = 2,12; $P \leq 0.0001$) (Figure 4.6.B). In the spring, the conspecific pairing of *H. axyridis* showed the greatest average separation, followed by *L. nigrinus* conspecifics and all heterospecific pairings, which were similar. During the summer evaluation, *H. axyridis* conspecifics once again maintained the greatest spatial separation, followed by the heterospecific pairing. The conspecific pairing of *S. tsugae* remained in closest proximity during each evaluation. Predator separation distances by time were not significantly different during spring ($F = 0.95$; df = 1,24; $P = 0.34$) or summer ($F = 1.91$; df = 1,12; $P = 0.19$).

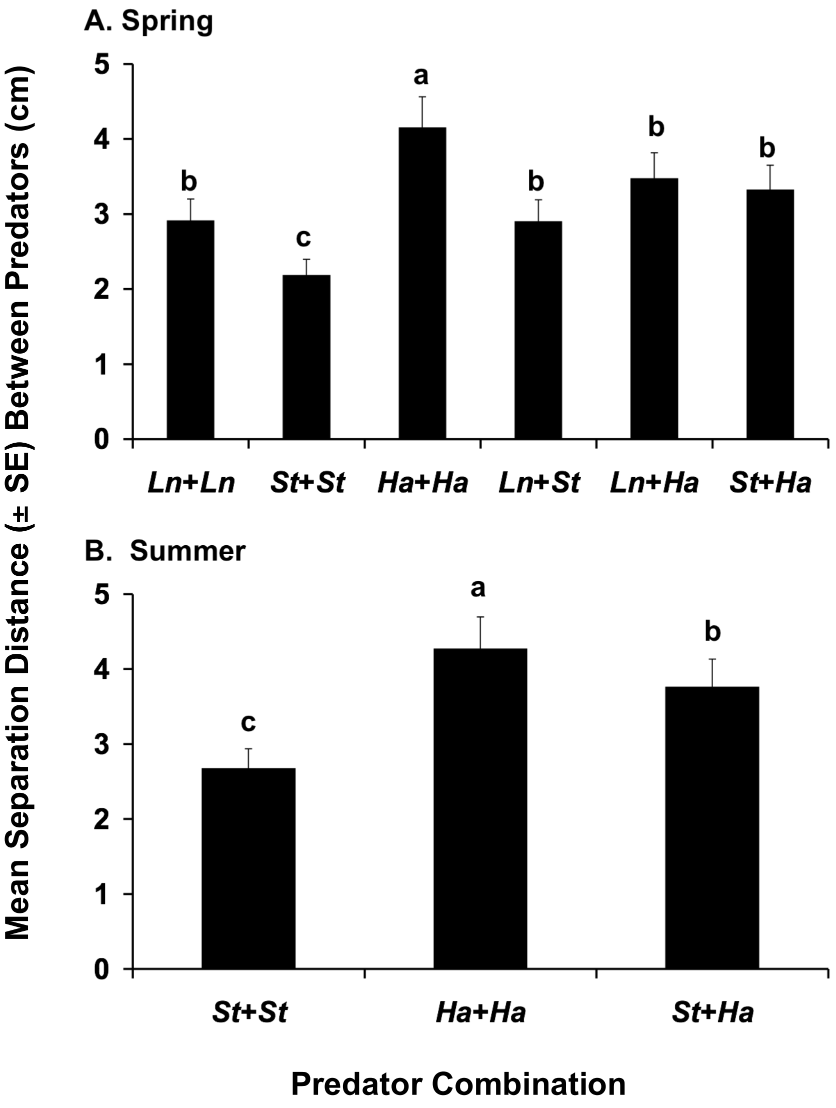

Figure 4.6. Mean separation distance (± SE) between adult female *L. nigrinus* (*Ln*), *S. tsugae* (*St*) and *H. axyridis* (*Ha*) in the spring (A) and summer (B) evaluation (n=6). Each species was evaluated in combination with one conspecific or one heterospecific predator. One-minute video segments were captured every 15 min over 24 h (≈ 96 observations). Means with the same letter were not significantly different (P ≥ 0.05).

Discussion

The results from these studies indicate that predator behavior in these species is quite variable. Searching by aphidophagous predators does not usually occur randomly, but involves two patterns of movement. Extensive searching uses fast linear movements between prey patches, while intensive searching includes slower and more directed movements that are induced by the perception of prey cues (Bell 1990). Searching may also change following prey capture, as the local area is subjected to more careful examination. Previous studies of *H. axyridis* indicate that this species uses vision and olfaction to find prey (Obata 1997, Harmon et al. 1998, Han and Chen 2002), and are consistent with the observations of this species with *A. tsugae*. Olfaction may also be used in prey finding by *L. nigrinus*, which possess antennae with several olfactory receptors (Broeckling and Salom 2003), and may be able to detect hemlock or *A. tsugae* volatiles. Field studies indicate that *L. nigrinus* can locate *A. tsugae* at very low population densities in its native range (Mausel 2005), suggesting the use of long-range prey perception ability. *Sasajiscymnus tsugae* possess a less sophisticated antennal morphology, with only a few olfactory receptors (Broeckling and Salom 2003), and thus may rely more on direct contact cues to locate prey, as was observed in this study.

The differences in intensive searching behavior in these species are likely related to predator feeding and reproductive biology. For *L. nigrinus*, greater prey evaluation may be a product of their close association with *A. tsugae* (Zilahi-Balogh et al. 2002). Since eggs are laid directly in adelgid ovisacs, it would be advantageous for females to insure that each ovisac is of sufficient size or quality for the successful development of progeny. Similarly, rearing studies indicate that *S. tsugae* is sensitive to prey quality (Palmer and Sheppard 2002), which may explain its intensive searching behavior, and more thorough prey evaluation, once prey is contacted. In contrast, *H. axyridis* may not be adapted to detect differences in the quality of *A. tsugae*, and intensive searching in this species appears to be directed toward areas of high prey density that are within close proximity. The results presented here are consistent with previously described searching behavior by this species with aphid prey in Japan (Obata 1986, Osawa 2000). In each case, it appeared that visual and olfactory cues were used extensively to guide movements to prey.

Variations in feeding and oviposition behaviors are also consistent with what is known regarding predator biology and foraging ecology. For *L. nigrinus*, reduced disruption of the ovisac during feeding may be advantageous, as it provides increased protection for its progeny as they develop within the ovisac. Microscopic examinations at the conclusion of each trial confirmed that feeding on *A. tsugae* by *L. nigrinus* was often limited, and this may serve as a way to further evaluate prey quality. In contrast, feeding by *S. tsugae* and *H. axyridis* was more disruptive to the ovisac, and this may actually better facilitate entry by progeny that emerge at nearby branch locations. For oviposition behavior, the antennal and abdominal contact by *L. nigrinus* on the surface of the ovisac may provide an additional assessment of prey quality. It may also assist in the detection of conspecific chemical markers or eggs, allowing females to reduce the frequency of intraspecific interactions, in which competition for prey or cannibalism may occur among progeny. Oviposition location may be related to reducing interspecific competition, as eggs of *L. nigrinus* and *S. tsugae* are vulnerable to predation by all three species (Chapter 2), and placement in more concealed locations may lower the risk of predation or provide a higher degree of buffering against environmental conditions. In contrast, the apparency of *H. axyridis* eggs appears to be due to the presence of chemical deterrents (Ayer and Brown 1977, Hemptinne et al. 2000, Agarwala and Yasuda 2001), and may serve to increase detection by conspecifics and reduce levels of intraspecific competition in this species. Variations in resting behavior followed a similar pattern, with *L. nigrinus* and *S. tsugae* being located in more concealed locations and *H. axyridis* maintaining greater apparency. Generalist predators often engage in intraguild predation when prey becomes scarce (Yasuda and Shinya 1997, Burgio et al. 2002); therefore, more concealed resting locations may provide additional protection for the specialist species.

Daily predator activity for these species can be generally classified as continuous, in which shorter searching, feeding and oviposition events are punctuated by longer periods of rest. There were no apparent temporal patterns for these species, in agreement with previous daily activity studies of *H. axyridis* (Obata and Johki 1990). In the spring, increased activity and a more even behavior distribution by *L. nigrinus* was likely due to this species being highly cold adapted (Lamb et al. 2005a, Zilahi-Balogh et al. 2003c). Because behavior in these species is influenced by temperature (Cheah and McClure 1998, Stathas et al. 2001, Zilahi-Balogh et al. 2002), the developmental threshold of *L. nigrinus*, which is lower than *S. tsugae* or *H. axyridis* by 5.8 and 7.5ºC (Cheah and McClure 1998, LaMana and Miller 1998, Zilahi-Balogh et al. 2003b), allowed this species to maintain greater activity levels. Consistent with this result, in the summer evaluation, *S. tsugae* and *H. axyridis* had a more even behavioral distribution as temperatures increased. However, even when temperatures were similar to optimal rearing conditions for the coccinellids, oviposition may still have been limited due to experimental constraints such as low prey quality (Palmer and Sheppard 2002), host tree health (Sheppard and Palmer 2004) nutrient limitation, or the inability to disperse to potentially more favorable microhabitats.

Conspecific predators exerted influence on the behavior patterns of *L. nigrinus* and *H. axyridis*, while conspecific effects were not apparent for *S. tsugae*. In the spring, the addition of *L. nigrinus* conspecifics increased searching behaviors and decreased resting, while feeding and oviposition remained similar. This response is likely related to prey selectivity, as females had to spend more time to locate acceptable oviposition sites. If behaviors in this species were altered in response to passive chemical cues associated with conspecific contact of prey or branch substrates, we would expect feeding and oviposition rates to be affected also; however, they were not. This also indicates that females may be highly adapted to detect the presence of eggs or oviposition-deterring chemical cues of conspecifics. The wide-ranging effects of conspecifics on *H. axyridis* behavior are consistent with previous studies of the chemical ecology of this predator. Intraspecific interactions in this species are regulated by both passive substrate marking using fecal cues (Agarwala et al. 2003) and actively deposited oviposition-deterring pheromones (Yasuda et al. 2000). Given the temporary nature of many prey populations and its ability to cannibalize (Osawa 1993, Hironori and Katsuhiro 1997, Burgio et al. 2002), selective pressure would favor avoiding areas already occupied by conspecifics, consistent with the results observed in these trials. Conspecific effects for *S. tsugae* may have been masked by the predominance of resting behavior in these trials, as previous studies indicate that this species has the ability to cannibalize (McClure 1995), and its behavior may therefore be regulated by similar mechanisms. In contrast, temporal activity patterns of all species were not significantly altered by heterospecifics, indicating that these species may be compatible with *A. tsugae* and suggesting that these predators may not be adapted to detect the chemical cues of one another.

Relative to assay size, all predator pairings appeared to maintain a high degree of spatial separation. While it is difficult to assess the responses due to the limited size and architecture of the assay, it suggests that avoidance behaviors may occur. Distances were greatest between conspecifics of *H. axyridis* and *L. nigrinus*, and this may again be in response to chemical cues, as has been documented in similar predator studies (Grostal and Dicke 1999). The shorter separation distances for *S. tsugae* conspecifics were likely influenced again by the predominance of resting behavior, during which time conspecifics were often found in close proximity. In contrast, separation distances between *S. tsugae* conspecifics were similar to that of the other species during searching and feeding events. Heterospecific pairings also exhibited high spatial separation within the context of these assays. Overall, interspecific effects on spatial patterns appeared be in response to tactile cues (direct contact), rather than chemical cues. Video evidence consistently showed that predator disturbance and re-location was high in response to direct contact, particularly by the specialists when contacted by *H. axyridis*. Avoidance behaviors and changes in spatial patterns in response to heterospecifics have been shown to occur in other aphidophagous predator guilds (Musser and Shelton 2003, Sato et al. 2005), and may regulate spatial relationships in this system as well.

Overall, inferences based on these observations are limited due to the experimental design and duration, which may have exerted a great deal of influence on these species. Video recordings of predator behavior were difficult to obtain using a single camcorder due to the architecture of the hemlock clippings, which often shielded predators from view. In addition, video quality was limited by using a standard camcorder, and a more sophisticated design using a model with greater specialization may assist in better documenting individual behaviors in these species. Predators also displayed a high degree of plasticity in individual behaviors, and previous studies indicate that searching patterns can change in response to prey type, and that behavior may be conditioned in some coccinellids with continuous rearing on a single prey (Ettifouri and Ferran 1993). In addition, the techniques necessary to study predator behavior in detail do not allow for the immigration and emigration that may occur on a larger scale in response to prey abundance or intraspecific and interspecific predator cues. However, daily activity patterns for *H. axyridis*, documented in field studies in Japan (Obata and Johki 1990), were very similar to those found for this species using the techniques presented here. Additional examinations of these predator species under field conditions are necessary to corroborate descriptions of predator behavior and daily activity. Overall, our studies suggest that these species are compatible, as temporal activity patterns were not highly coordinated, and predator avoidance responses may occur such that these species will maintain a high degree of spatial separation under more natural conditions.

Chapter 5

Conclusions

The presence of *H. axyridis* in hemlock stands infested with *A. tsugae* is well documented (Wallace and Hain 2000) and is likely to continue given that prey densities are high, and that native species are not reducing them substantially. As such, *H. axyridis* may be a formidable competitor to the newly introduced specialist predators, *L. nigrinus* and *S. tsugae*, as it is highly adapted for competition with other species (Yasuda et al. 2004, Cottrell 2005). Competitive interactions with generalist predators are often asymmetric, and can lead to interspecific interference with more specialized species (Polis et al. 1989, Lucas et al. 1998). With its greater size and voracity, *H. axyridis* has the potential to contribute to the decline or dispersal of *L. nigrinus* and *S. tsugae* from *A. tsugae* infestations through direct or indirect means. When examining competitive effects, it is useful to compare interspecific interference relative to intraspecific interference to provide an indication of risk for new species combinations (Lynch et al. 2001). In the laboratory and field studies presented here, interspecific interference among these species was never significantly greater than intraspecific, suggesting that *L. nigrinus*, *S. tsugae* and *H. axyridis* may be compatible in this system.

Overall, the lack of strong seasonal synchrony among these species may prove to limit levels of competition in the field. The seasonal overlap of *L. nigrinus* with *S. tsugae*, *H. axyridis* and native generalists may be limited because of their early spring development and adaptation to colder temperatures. However, *S. tsugae* may be at greater risk due to their higher temperature requirements and summer development that overlaps with other generalist predators such as lacewings and spiders, which can feed on immature life stages of this species (Casagrande et al. 2002). However, the risk to *S. tsugae* may be reduced due to the high level of *A. tsugae* currently available at release locations. In addition, the greater nutritional requirements of many generalists, including *H. axyridis*, may limit their duration in infested hemlock stands (Dixon 2000, Soares et al. 2004). Additional field studies of seasonal occurrence and population densities of predators of *A. tsugae* are needed and will allow for a better conceptual and ecological framework for examining and understanding the role of predators in the biological control program for *A. tsugae*.

In conclusion, these studies indicate that it will be advantageous to use multiple predator combinations over single species when implementing biological control for *A. tsugae*. Interspecific interference among these species did not occur to a significant degree in Petri dish assays in the laboratory or branch enclosures in the field. Similarly, video studies indicated that temporal and spatial patterns were not highly coordinated in these species, such that significant competitive interference is unlikely to occur under natural conditions. However, intraspecific interference negatively impacted predator effectiveness and should be considered in release strategies. The use of low-density releases is therefore recommended to reduce the potential negative effects of intraspecific competition.

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Further Study

Additional research projects that would complement the studies presented here include: 1) Examining the modes of intraspecific interference among these predator species, 2) Documenting predator movement dynamics at release sites and distributions in established populations, and 3) Evaluating competitive interactions among recently discovered predators and existing species being used for biological control of *A. tsugae*. Conspecific cues, both chemical and tactile (direct contact) have not been investigated for the two specialist species nor has the role of avoidance responses in mediating predator interactions under natural conditions. Also, few studies have documented dispersal patterns of the specialist predators following release, their distributions at established sites, and the degree of emigration and immigration that occurs among all three species in infested hemlock stands. Lastly, newly discovered predator species, including *Laricobius spp.* from Japan and China and *Scymnus spp.* (Coleoptera: Coccinellidae) from China, are currently being evaluated for use in biological control of *A. tsugae*, and their interactions with the species described here and with existing native predators in the eastern United States is unknown.

Literature Cited

Agarwala, B. K. and H. Yasuda. 2001. Overlapping oviposition and chemical defense of eggs in two co-occurring species of ladybird predators of aphids. J. Ethol. 19: 47-53.

Agarwala, B. K., H. Yasuda and Y. Kajita. 2003. Effect of conspecific and heterospecific feces on foraging and oviposition of two predatory ladybirds: Role of fecal cues in predator avoidance. J. Chem. Ecol. 29: 357-376.

Ayer, W. A. and L. M. Brown. 1977. The ladybug alkaloids including synthesis and biosynthesis. Heterocycles 7: 685-707.

Battles, J. J., N. Cleavitt, T. J. Fahey, and R. A. Evans. 1999. Vegetation composition and structure in two hemlock stands threatened by the hemlock woolly adelgid. pp. 55-61. *In:* Proceedings: Symposium on Sustainable Management of Hemlock Ecosystems in eastern North America. K.A. McManus, K.S. Shields and D.R. Souto (eds.) USDA For. Serv., Durham, NH.

Baumgras, J.E., P. E. Sendak and D. L. Sonderman. 1999. Ring shake in eastern hemlock: frequency and relationship to tree attributes. pp. 156-160. *In:* Proceedings: Symposium on Sustainable Management of Hemlock Ecosystems in eastern North America. K.A. McManus, K.S. Shields and D.R. Souto (eds.) USDA For. Serv., Durham, NH.

Bell, W. J. 1990. Searching behavior patterns in insects. Ann. Rev. Entomol. 35: 447-467.

Blumenthal, E. M. 2002. *Pseudoscymnus tsugae* in Pennsylvania forests, pp. 166-169. *In:* Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium. B. Onken, R. Reardon and J. Lashomb (eds.), Rutgers University, East Brunswick, NJ.

Broeckling, C. D. and S. M. Salom. 2003. Antennal morphology of two specialist predators of hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). Ann. Entomol. Soc. Am. 96: 153-160.

Brown, M. W. and S. S. Miller. 1998. Coccinellidae (Coleoptera) in apple orchards of eastern West Virginia and the impact of invasion by *Harmonia axyridis*. Entomol. News 109: 143-151.

Burgio, G., F. Santi and S. Maini. 2002. On intra-guild predation and cannibalism in *Harmonia axyridis* (Pallas) and *Adalia bipunctata* L. (Coleoptera: Coccinellidae). Biol. Control 24: 110-116.

Butin, E., M. Montgomery, N. Havill and J. Elkinton. 2002. Pre-release host range assessment for classical biological controls: Experience with predators for hemlock woolly adelgid, pp. 205-213. *In*: Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium. B. Onken, R. Reardon and J. Lashomb (eds.), Rutgers University, East Brunswick, NJ.

Casagrande, R. A., M. Desanto, J. Dacey and A. Lambert. 2002. *Pseudoscymnus tsugae* for biological control of the hemlock woolly adelgid in suburban settings, pp. 170-180. *In*: Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium. B. Onken, R. Reardon and J. Lashomb (eds.), Rutgers University, East Brunswick, NJ.

Cheah, C. A. S. J. and M. S. McClure. 1998. Life history and development of *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae), a new predator of the hemlock wooly adelgid (Homoptera: Adelgidae). Environ. Entomol. 27: 1531-1536.

Cheah, C. A. S. J. and M.S. McClure. 2000. Seasonal synchrony of life cycles between the exotic predator, *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae) and its prey, hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). Agric. For. Entomol. 2: 241-251.

Cheah, C., M. Montgomery, S. Salom, B. Parker, S. Costa and M. Skinner. 2004. Biological control of hemlock woolly adelgid, pp. 22. R. Reardon and B. Onken (eds.). USDA For. Serv., Morgantown, WV.

Colunga-Garcia, M. and S. H. Gage. 1998. Arrival, establishment, and habitat use of the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in a Michigan landscape. Environ. Entomol. 27: 1574-1580.

Cottrell, T. E. 2005. Predation and cannibalism of lady beetle eggs by adult lady beetles. Biol. Cont. 34: 159-164.

Cottrell, T. E. and K. V. Yeargan. 1998. Intraguild predation between an introduced lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae) and a native lady beetle, *Coleomegilla maculata* (Coleoptera: Coccinellidae). J. Kans. Entomol. Soc. 71: 159-163.

Croft, B. A. and I. V. MacRae. 1992. Biological control of apple mites by mixed populations of *Metaseiulus occidentalis* Nesbitt and *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae). Environ. Entomol. 21: 202-209.

Dixon, A. F. G. 2000. Insect predator-prey dynamics: Ladybird beetles and biological control. Cambridge University Press, Cambridge, MA.

Ettifouri, M. and A. Ferran.1993. Influence of larval rearing diet on the intensive searching behavior of *Harmonia axyridis* (Col.: Coccinellidae) larvae. Entomophaga

38: 51-59.

Evans, R.A., E. Johnson, J. Shreiner, A. Ambler, B. Battles, N. Cleavitt, T. Fahey, J. Sciascia and E. Pehek. 1996. Potential impacts of hemlock woolly adelgid (*Adelges tsugae*) on eastern hemlock (*Tsuga canadensis*) ecosystems. pp. 42-57. *In*: Proceedings of the First Hemlock Woolly Adelgid Review. S.M. Salom, T.C. Tigner and R.C. Reardon (eds.). USDA For. Serv., Charlottesville, VA.

Evans, R. A. 2002. An ecosystem unraveling? pp. 23-33. *In*: Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium. B. Onken, R. Reardon, and J. Lashomb (eds.). Rutgers University, East Brunswick, NJ.

Fidgen, J. G., Q. C. McClellan and S. M. Salom. 2002. Efficacy and residual activity of two systemic insecticides for control of hemlock woolly adelgid on young eastern hemlocks, pp. 329-333. *In*: Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium. B. Onken, R. Reardon and J. Lashomb (eds.). Rutgers University, East Brunswick, NJ.

Foster, D.R. 1999. Hemlock's future in the context of its history: an ecological perspective. pp. 1-4. *In*: Proceedings: Symposium on sustainable management of hemlock ecosystems in eastern North America. K. A. McManus, K. S. Shields, and D. R. Souto (eds.). USDA For. Serv., Durham, NH.

Frazer, B. D., N. Gilbert, V. Nealis and D. A. Raworth. 1981. Control of aphid density by a complex of predators. Can. Entomol. 113: 1035-1041.

Furniss, R. L. and V. M. Carolin. 1977. Western Forest Insects. USDA For. Serv. Misc. Pub. 1339.

Gordon, R. D. 1985. The Coccinellidae (Coleoptera) of America north of Mexico. J. New York Entomol. Soc. 93: 1-912.

Gray, D. R. and S. M. Salom. 1996. Biology of the hemlock woolly adelgid in the southern Appalachians. pp. 26-32. *In*: Proceedings of the First Hemlock Woolly Adelgid Review. S. M. Salom, T. C. Tigner, and R. C. Reardon (eds.). USDA For. Serv. Charlottesville, VA.

Grostal, P. and M. Dicke. 1999. Direct and indirect cues of predation risk influence behavior and reproduction of prey: A case for acarine interactions. Behav. Ecol. 10: 422-427.

Hain, F.P. 1989. The balsam woolly adelgid in North America. pp. 88-109. *In*: Dynamics of Forest Insect Populations: Patterns, Causes, Implications. A. A. Berryman (ed.). Plenum Pub. Corp., New York, NY.

Han B. Y and Z. M. Chen. 2002. Composition of volatiles from intact and tea-aphid damaged tea shoots and their allurement to several natural enemies of the tea aphid. J. Appl. Entomol. 126: 497-500.

Harmon, J. P., J. E. Losey and A. R. Ives. 1998. The role of vision and color in the close proximity foraging behavior of four coccinellid species. Oecologia 115: 287-292.

Havill, N.P., M.E. Montgomery, G. Yu, S. Shiyake and A. Caccone. 2006. Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. Ann. Entomol. Soc. Am. 99: 195-203.

Hemptinne, J. L., G. Lognay, C. Gauthier and A. F. G. Dixon. 2000. Role of surface chemical signals in egg cannibalism and intraguild predation in ladybirds (Coleoptera: Coccinellidae). Chemoecology 10: 123-128.

Hironori, Y. and S. Katsuhiro. 1997. Cannibalism and interspecific predation in two predatory ladybirds in relation to prey abundance in the field. Entomophaga 42: 153-163.

Iperti, G. and E. Bertrand. 2001. Hibernation of *Harmonia axyridis* (Coleoptera: Coccinellidae) in southeastern France. Acta Soc. Zool. Bohem. 65: 207-210.

Inouyue, M. 1945. Monographische studie uber die Janischen koniferen-gallenlause (Adelgidae). Bull. Sapporo Branch Govt. Forest Exp. Sta. 15: 1-105.

Koch, R. L. 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its biology, uses in biological control, and non-target impacts. J. Insect Sci. 3: 1-16.

Koch, R. L. and W. D. Hutchinson 2003. Phenology and blacklight trapping of the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in a Minnesota agricultural landscape. J. Entomol. Sci. 38: 477-480.

Lamana, M. L. and J. C. Miller. 1996. Field observations on *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in Oregon. Biol. Control 6: 232-237.

Lamana, M. L. and J. C. Miller. 1998. Temperature dependent development in an Oregon population of *Harmonia axyridis* (Coleoptera: Coccinellidae). Environ. Entomol. 27: 1001-1005.

Lamb, A. B., S. M. Salom and L. T. Kok. 2002. Field evaluation and improvement of rearing procedures for *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), a predator of hemlock woolly adelgid, pp. 189-196. *In*: Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium. B. Onken, R. Reardon and J. Lashomb (eds.). Rutgers University, East Brunswick, NJ.

Lamb, A. B., S. M. Salom and L. T. Kok. 2005a. Survival and reproduction of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), a predator of hemlock woolly adelgid *Adelges tsugae* Annand (Homoptera: Adelgidae) in field cages. Biol. Control 32: 200-207.

Lamb, A. B., S. M. Salom and L. T. Kok. 2005b. Guidelines for rearing *Laricobius nigrinus* Fender, pp. 309-318. *In*: Proceedings: Third Symposium on Hemlock Woolly Adelgid in the Eastern United States. B. Onken and R. Reardon (eds.). USDA For. Serv., Asheville, NC.

Larock, D. R. and J. J. Ellington. 1996. An integrated pest management approach, emphasizing biological control, for pecan aphids. Southwest. Entomol. 21: 153-167.

Lawrence, J. F. and T. F. Hlavac. 1979. Review of the Derodontidae (Coleoptera: Polyphaga) with new species from North America and Chile. Coleopt. Bull. 33: 369-414.

Lucas, E., D. Coderre and J. Brodeur. 1998. Intraguild predation among aphid predators: characterization and influence of extraguild prey density. Ecology 79: 1084-1092.

Lynch, L. D., H. M. T. Hokkanen, D. Babendreier, F. Bigler, G. Burgio, Z. H. Gao, S. Kuske, A. Loomans, I. Menzler-Hokkanen, M. B. Thomas, G. Tommasini, J. K. Waage, J. Lenteren and Q. Zeng. 2001. Insect biological control and non-target effects: a European perspective, pp. 99-125. *In*: Evaluating indirect ecological effects of biological control. E. Wajnberg, J. K. Scott and P. C. Quinby (eds.). CABI Publishing, Wallingford, UK.

Matsuka, M. and K. Niijima. 1985. *Harmonia axyridis*. *In*: Singh, P. and R. F. Moore (eds.), Handbook of Insect Rearing, Vol. I., Tokyo, Japan.

Mausel, D. L. 2004. Establishment and sampling of *Laricobius nigrinus*, pp. 16. *In*: Hemlock Woolly Adelgid Biological Control Committee Meeting. R. Reardon and B. Onken (eds.). USDA For. Serv., Annapolis, MD.

Mausel, D. L. 2005. Observations on fecundity and natural enemies of hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) in Seattle, Washington. Pan-Pac. Entomol. 81: 97-98.

Mayer, M., R. Chianese, T. Scudder, J. White, K. Vongpaseuth and R. Ward. 2002. Thirteen years of monitoring the hemlock woolly adelgid in New Jersey forests, pp. 50-60. *In* Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium. B. Onken, R. Reardon and J. Lashomb (eds.). Rutgers University, East Brunswick, NJ.

McClure, M. S. 1987. Biology and control of hemlock woolly adelgid, pp. 3-9.

Connecticut Agricultural Experiment Station, New Haven, CT.

McClure, M. S. 1989. Evidence of a polymorphic life cycle in the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). Ann. Entomol. Soc. Am. 82: 50-54.

McClure, M. S. 1990. Role of wind, birds, deer, and humans in the dispersal of hemlock woolly adelgid (Homoptera: Adelgidae). Environ. Entomol. 19: 36-43.

McClure, M. S. 1991a. Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae). Environ. Entomol. 19: 36-43.

McClure, M. S. 1991b. Control of hemlock woolly adelgid. Yankee Nursery Quart. 1: 1-3.

McClure, M. S. 1992a. Effects of implanted and injected pesticides and fertilizers on the survival of *Adelges tsugae* (Homoptera: Adelgidae) and on the growth of *Tsuga canadensis*. J. Econ. Entomol. 85: 468-472.

McClure, M.S. 1992b. Hemlock woolly adelgid. American Nurseryman. 176: 82-86.

McClure, M.S. 1995. Managing hemlock woolly adelgid in ornamental landscapes. Bull. Conn. Agric. Exp. Sta. 925.

McClure, M. S. and C. A. S. J. Cheah. 1999. Reshaping the ecology of invading populations of hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae), in eastern North America. Biol. Invasions 1: 247-254.

McClure, M. S., C. A. S. J. Cheah and T. C. Tigner. 1999. Is *Pseudoscymnus tsugae* the solution to the hemlock woolly adelgid problem: an early perspective. pp. 89-96. *In*: Proceedings: Symposium on Sustainable Management of Hemlock Ecosystems in eastern North America. K.A. McManus, K.S. Shields and D.R. Souto (eds.). USDA For. Serv., Durham, NH.

McClure, M.S., S. M. Salom and K. S. Shields. 2001. Hemlock woolly adelgid, pp. 14. USDA For. Serv., FHTET 01-03.

McWilliams, W. H. and T. L. Schmidt. 1999. Composition, structure, and sustainability of hemlock ecosystems in eastern North America. pp. 5-10. *In*: Proceedings: Symposium on Sustainable Management of Hemlock Ecosystems in eastern North America. K.A. McManus, K.S. Shields and D.R. Souto (eds.). USDA For. Serv., Durham, NH.

Michaud, J. P. 2002. Invasion of the Florida citrus ecosystem by *Harmonia axyridis* (Coleoptera: Coccinellidae) and asymmetric competition with a native species, *Cycloneda sanguinea*. Environ. Entomol. 31: 827-835.

Michaud, J. P. and A. K. Grant. 2004. Adaptive significance of sibling egg cannibalism in Coccinellidae: comparative evidence from three species. Ann. Entomol. Soc. Am. 97: 710-719.

Montgomery, M. E. and S. M. Lyon. 1996. Natural enemies of adelgids in North America: their prospect for biological control of *Adelges tsugae* (Homoptera: Adelgidae), pp. 89-102. *In*: Proceedings of the First Hemlock Woolly Adelgid Review. S. M. Salom, T. C. Tigner and R. C. Reardon (eds.). USDA For. Serv., Charlottesville, VA.

Murdoch, W. W. 1990. The relevance of pest-enemy models to biological control, pp. 1-24. *In*: Critical issues in biological control. M. Mackauer, L. E. Ehler and J. Roland (eds.). Intercept, Andover, UK.

Musser, F. R. and A. M. Shelton. 2003. Factors altering the temporal and within-plant distribution of coccinellids in corn and their impact on potential intraguild predation. Environ. Entomol. 32: 575-583.

Obata, S. 1986. Mechanisms of prey finding in the aphidophagous ladybird beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae). Entomophaga 31: 301-311.

Obata, S. 1997. The influence of aphids on the behaviour of adults of the ladybird beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae). Entomophaga 24: 103-106.

Obata, S. and Y. Johki. 1990. Distribution and behaviour of adult ladybird, *Harmonia axyridis* (Coleoptera: Coccinellidae), around aphid colonies. Jap. J. Entomol. 58: 839-845.

Orwig, D. A. 2002. Stand dynamics associated with chronic hemlock woolly adelgid infestation in southern New England pp. 36-46. *In*: Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium. B. Onken, R. Reardon and J. Lashomb (eds.). Rutgers University, East Brunswick, NJ.

Orwig, D. A. and D. R. Foster. 1998. Forest response to the introduced hemlock woolly adelgid in southern New England USA. J. Tor. Bot. Soc. 125: 60-73.

Osawa, N. 1992. Sibling cannibalism in the lady beetle *Harmonia axyridis*: fitness consequences for mother and offspring. Res. Popul. Ecol. 34: 45-55.

Osawa, N. 1993. Population field studies of the aphidophagous ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae): life tables and key factor analysis. Res. Popul. Ecol. 35: 335-348.

Osawa N. 2000. Population field studies of the aphidophagous ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae): resource tracking and population characteristics. Popul. Ecol. 42: 115-127.

Palmer, D. and J. Sheppard. 2002. Mass rearing *Pseudoscymnus tsugae*, a predator of hemlock woolly adelgid, at the New Jersey Department of Agriculture: Challenges and lessons, pp. 214-220. *In*: Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium. B. Onken, R. Reardon and J. Lashomb (eds.). Rutgers University, East Brunswick, NJ.

Parker, B. L., M. Skinner, S. Gouli, T. Ashikaga and H. B. Teillon. 1999. Low lethal temperature for hemlock woolly adelgid (Homoptera: Adelgidae). Environ. Entomol. 28: 1085-1091.

Pasteels, J. M., C. Deroe, B. Tursch, J. C. Braekman, D. Daloze, and C. Hootele. 1973. Distribution et activites des alcaloides defensifs des Coccinellidae. J. Insect Physiol. 19: 1771-1784.

Polis, G. A., C. A. Myers and R. Holt. 1989. The evolution and ecology of intraguild predation: competitors that eat each other. Ann. Rev. Ecol. Syst. 20: 297-330.

Quimby, J. W. 1996. Value and importance of hemlock ecosystems in the eastern United States. pp. 1-8. *In*: Proceedings of the First Hemlock Woolly Adelgid Review. S. M. Salom, T. C. Tigner and R. C. Reardon (eds.). USDA For. Serv., Charlottesville, VA.

Reid, W., B. L. Parker, M. Skinner, S. Gouli and H. B. Teillon. 2002. Insect-killing fungi for management of hemlock woolly adelgid: A review of progress, pp. 198-204. *In*: Proceedings: Hemlock Woolly Adelgid in the Eastern United States Symposium. B. Onken, R. Reardon and J. Lashomb (eds.). Rutgers University, East Brunswick, NJ.

Rhea, R. J. 1995. Preliminary results for the chemical control of hemlock woolly adelgid in ornamental and natural settings, pp. 113-125. *In*: Proceedings of the First Hemlock Woolly Adelgid Review. S. M. Salom, T. C. Tigner and R. C. Reardon (eds.). USDA For. Serv., Charlottesville, VA.

Rhoades, M. H. 1996. Key to first and second instars of six species of Coccinellidae (Coleoptera) from alfalfa in southwest Virginia. J. New York Entomol. Soc. 104: 83-88.

Rosenheim, J. A., L. R. Wilhoit, and C. A. Armer. 1993. Influence of intraguild predation among generalist insect predators on the suppression of an herbivore population. Oecologia 96: 439-449.

SAS Institute. 2001. SAS/STAT® User's manual, version 8.2. SAS Institute, Cary, NC.

Salom, S. M., A. A. Sharov, W. T. Mays and J. W. Neal. 2001. Evaluation of aestival diapause in hemlock woolly adelgid (Homoptera: Adelgidae). Environ. Entomol. 30: 877-882.

Sasaji, H. 1977. Larval characters of Asian species of the genus *Harmonia* Mulsant. Memoir of the Faculty of Education, Fukui University series II, Natural Science 27:1-17.

Sasaji, H. and M. S. McClure. 1997. Description and distribution of *Pseudoscymnus tsugae* sp. nov. (Coleoptera: Coccinellidae), an important predator of hemlock woolly adelgid in Japan. Ann. Entomol. Soc. Am. 90: 563-568.

Sato, S., Y. Hironori and E. W. Evans. 2005. Dropping behavior of larvae of aphidophagous ladybirds and its effects on incidence of intraguild predation: interactions between the intraguild prey, *Adalia bipunctata* (L.) and *Coccinella septempunctata* (L.), and the intraguild predator, *Harmonia axyridis* Pallas. Ecol. Entomol. 30: 220-224.

Sheppard, J. L., and D. J. Palmer. 2004. Host tree may affect *Pseudoscymnus tsugae* reproduction, pp. 6. *In*: Hemlock Woolly Adelgid Biological Control Committee Meeting. R. Reardon and B. Onken (eds.). USDA Forest Service FHTET 04-08, Annapolis, MD.

Skinner, M., B. L. Parker, S. Gouli and T. Ashikaga. 2003. Regional responses of hemlock woolly adelgid (Homoptera: Adelgidae) to low temperatures. Environ. Entomol. 32: 523-528.

Snyder, W. E., S. B. Joseph, R. F. Preziosi, and A. J. Moore. 2000. Nutritional benefits of cannibalism for the lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae) when prey quality is poor. Environ. Entomol. 29: 1173-1179.

Soares, A.O., D. Coderre and H. Schanderl. 2004. Dietary self-selection behavior by the adults of the aphidophagous lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae). J. Animal Ecol. 73: 478-486.

Stathas, G. J., P. A. Eliopoulos, D. C. Kontodimas, and J. Giannopapas. 2001. Parameters of reproductive activity in females of *Harmonia axyridis* (Coleoptera: Coccinellidae). Eur. J. Entomol. 98: 547-549.

Takahashi, K. 1987. Differences in oviposition initiation and sites of lady beetles, *Coccinella septempunctata bruckii* Mulsant and *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in the field. Jpn. J. Appl. Entomol. Zool. 31: 253-254.

Wagner, J. D., M. D. Glover, J. B. Moseley and A. J. Moore. 1999. Heritability and fitness consequences of cannibalism in *Harmonia axyridis*. Evol. Ecol. Res. 1: 375-378.

Wallace, M. S. and F. P. Hain. 2000. Field surveys and evaluation of native and established predators of the hemlock woolly adelgid (Homoptera: Adelgidae) in the southeastern United States. Environ. Entomol. 29: 638-644.

Ward, J.S., M. E. Montgomery, C. A. S. J. Cheah, B. P. Onken and R. S. Cowles. 2004. Eastern Hemlock Forests: Guidelines to Minimize the Impacts of Hemlock Woolly Adelgid. USDA For. Serv., NA-TP-03-04.

Webb, R. E., J. R. Frank, and M. J. Raupp. 2003. Eastern hemlock recovery from hemlock woolly adelgid damage following imidacloprid therapy. J. Arbor. 29: 298-302.

Wilson, E. O. 1971. The Insect Societies. 548 pp. Harvard University Press, Cambridge, MA.

Yasuda, H. and K. Shinya. 1997. Cannibalism and interspecific predations of lady beetles in spring alfalfa fields. Jpn. J. Entomol. 57: 199-203.

Yasuda, H., and N. Ohnuma. 1999. Effect of cannibalism and predation on the larval performance of two ladybird beetles. Entomol. Exp. Appl. 93: 63-67.

Yasuda, H., T. Takagi, and K. Kogi. 2000. Effects of conspecific and heterospecific larval tracks on the oviposition behavior of the predatory ladybird, *Harmonia axyridis* (Coleoptera: Coccinellidae). Eur. J. Entomol. 97: 551-553.

Yasuda, H., E. W. Evans, Y. Kajita, K. Urakawa, T. Takizawa. 2004. Asymmetric larval interactions between introduced and indigenous ladybirds in North America. Oecologia 141: 722-731.

Young, R. F., K. S. Shields, and G. P. Berlyn. 1995. Hemlock woolly adelgid (Homoptera: Adelgidae): stylet bundle insertion and feeding sites. Ann. Entomol. Soc. Amer. 88: 827-835.

Zar, J. H. 1998. Biostatistical Analysis. 4th edition. Prentice-Hall, Englewood Cliffs, NJ.

Zilahi-Balogh, G. M. G., L. T. Kok, and S. M. Salom. 2002. Host specificity of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), a potential biological control agent of the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). Biol. Control 24: 192-198.

Zilahi-Balogh, G. M. G., S. M. Salom and L. T. Kok. 2003a. Development and reproductive biology of *Laricobius nigrinus*, a potential biological control agent of *Adelges tsugae.* BioControl 48: 293-306.

Zilahi-Balogh, G. M. G., S. M. Salom and L. T. Kok. 2003c. Temperature-dependent development of the specialist predator *Laricobius nigrinus* (Coleoptera: Derodontidae). Environ. Entomol. 32: 1322-1328.

Zilahi-Balogh, G. M. G., L. M. Humble, A. B. Lamb, S. M. Salom and L. T. Kok. 2003b. Seasonal abundance and synchrony between *Laricobius nigrinus* (Coleoptera: Derodontidae) and its prey, the hemlock woolly adelgid (Homoptera: Adelgidae) in British Columbia. Can. Entomol. 135: 103-115.