Assessing performance and compatibility of three *Laricobius* species as predators of

hemlock woolly adelgid, *Adelges tsugae*.

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Assessing performance and compatibility of three predators of hemlock woolly adelgid

from the genus *Laricobius*

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ABSTRACT

Predation, egg production and survivorship of *Laricobius nigrinus* Fender*, L. rubidus,* LeConte, and *L. osakensis* Montgomery and Shiyaki (proposed), predators of hemlock woolly adelgid, *Adelges tsugae,* Annand, were investigated in the laboratory and in the field. In individual assays, *L. rubidus* oviposited fewer eggs than either *L. nigrinus* or *L. osakensis.* In assays containing congeneric or conspecific groups of adult *Laricobius*, *L. osakensis* preyed upon the greatest number of ovisacs. When all three species were together, the numbers of ovisacs preyed upon were similar to the mean of all three individual species. Adult predators fed on few eggs and did not exhibit any species preference. The numbers of *A. tsugae* ovisacs fed upon did not differ significantly by groups of congeneric or conspecific *Laricobius* larvae. *Laricobius* adults and larvae had high survival rates throughout all experiments. In the field, predators were enclosed in sleeve cages with both high (> 120 ovisacs) and low (< 90 ovisacs) *A. tsugae* densities for 1 wk. All branches with caged beetles had significantly greater numbers of ovisacs preyed upon than branches caged without beetles. No differences in predation or egg production were found among the conspecific and congeneric groupings. Predation was uniformly higher at the high prey-density than at the low prey-density. Survivorship among predators did not differ significantly at either prey density. Results from both laboratory and field experiments suggest that these species are able to co-exist and can be in the same location for biological control of *A. tsugae.*

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Introduction and Literature Review

1.1 *Adelges tsugae* Overview

Hemlock woolly adelgid (HWA), *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 U.S.A. *A. tsugae* in the eastern United States is believed to have

originated from Japan where it poses no threat to native *Tsuga* species (Havill et al. 2006). First

discovered in 1951 near Richmond, Virginia, it has since spread throughout a large portion of the

hemlock range causing widespread mortality in the eastern U.S. (Cheah et al. 2004). Hemlock in

the eastern U.S. plays many important roles, both environmentally and commercially. It is a

dominant climax species occupying 7.7 million ha of forested land, is shade tolerant, and creates

unique microclimates that support many terrestrial and aquatic species (Evans et al. 1996,

Schmidt and McWilliams 1996, Ward et al. 2004). Hemlocks comprise 22 percent of the total

volume of softwood grown in the northeastern United States. Many hemlock cultivars are often

planted in ornamental settings where tree mortality may detract from private and public property

values (Ward et al. 2004, Battles et al. 1999). As *A. tsugae* began spreading throughout the

native hemlock range in the eastern United States, the economic and environmental impacts

became apparent. Due to damage inflicted upon the two native hemlocks by this invasive pest

and the lack of effective management strategies, resource managers began to seek new and

innovative ways to protect and preserve both tree species. One such innovation is the attempt to

develop resistant eastern hemlock hybrids (Lagalante and Montgomery 2003, Bentz et al. 2005,

Montgomery et al. 2005, Playfoot and Ward 2005). Another is conservation of germplasm. Germplasm conservation is being carried out due to the potential extinction of Carolina hemlock (Tighe et al. 2005). *A. tsugae* is capable of adapting to very cold climates and will likely continue to spread throughout much of the eastern hemlock range (Butin et al. 2005); however, extreme winter cold could limit expansion into northern New England (Parker et al. 1999, Gouli et al. 2000, Evans et al. 2007).

1.2 The *A. tsugae* Lifecycle

A. tsugae in its native habitat is a holocyclic species that includes a sexual generation with accompanying alternating parthenogenesis. The sexual generation occurs on a primary host of spruce *(Picea* spp.) and the parthenogenetic generation occurs on a secondary host of *Tsuga* species (Havill and Footit 2007). In the U.S. the life cycle is anholocyclic, or parthenogenetic, and occurs only on its secondary host, *Tsuga*, of which nine species exist worldwide (Farjon 1990). The primary host, tiger-tail spruce, *Picea polita* (Siebold and Zucc.) does not grow in North America (Havill and Footit 2007, McClure 1989, Blackman and Eastop 1994). Havill et al. (2006) examined the mitochondrial DNA of *A. tsuage* populations and concluded that the origin for the eastern North American population of *A. tsuage* is Osaka, Japan. Adelgids as a group are highly host-specific and feed on only specific genera of the Pinaceae (Havill and Footit 2007).

A. tsugae has an overwintering generation, termed sistentes that is active from summer to early spring. Adults of the sistentes generation produce 50-300 eggs per individual in woolly ovisacs from March to May and adults of a second generation, the progredientes, produce up to 75 eggs from May to June (Zilahi-Balogh et al. 2003, McClure 1989, 1991). Eggs of the sistentes

generation hatch into crawlers from April to May (McClure 1991). The developing crawlers form two groups, the progredientes that are asexual and remain on hemlock, and occasionally a winged form, termed sexupara, representing the sexual generation (McClure 1989, Gray and Salom 1996). The number of sexupara produced yearly is a density-dependent response to poor host quality and it serves as a population regulation mechanism (McClure 1991). Sexuparae fly in search of the non-existent primary host and represent a dead-end generation in North America. Crawlers of the sistentes generation settles on hemlock, aestivate through the summer, complete development in the fall/winter, and lay progredientes eggs in the late winter/early spring (McClure 1987, McClure 1989, Gray and Salom 1996, Zilahi-Balogh et al. 2003). First instar crawlers of both generations disperse by crawling on birds and other forest animals during April-July (McClure 1990) or are dispersed by wind (Evans and Gregoire 2007). *A. tsugae* eggs may be spread when an ovisac's sticky flocculence becomes attached to the fur or feathers of animals. Areas that contain hiking trails or riparian areas often have high adelgid populations because both people and forest animals inadvertently disperse adelgids while moving through forested areas (Graham et al. 2005, Koch et al. 2006).

Hemlocks are damaged by the feeding activities of *A. tsugae*. Adelgid nymphs penetrate plant tissues with their stylets to feed on the parenchyma cells that serve as nutrient transfer and storage cells in the xylem rays (Young et al. 1995). Tree health deteriorates due the depletion of photosynthates, which then inhibits shoot growth and causes bud mortality, twig dieback, foliage discoloration and premature defoliation. *A. tsugae* will cause physiological damage once 30% of a trees shoots have been infested (Fidgen et al. 2006). Once the crown transparency reaches 60% tree mortality begins (Mayer et al. 2002). High populations of *A. tsugae* reduce new shoot

growth during the following growing season. This is followed by a reduction in pest density because *A. tsugae* performs poorly on older shoots (McClure 1991). Although both infested hemlock trees and *A. tsugae* populations generally resume growth after pest densities decline, the infested hemlock trees do not fully recover. Some trees may survive for decades in a cycle of decline and recovery while others may die only four years after the initial infestation occurs. Weakened trees are more susceptible to many environmental stressors such as drought, native insect attacks, and disease all eventually causing mortality (McClure 1991, Young et al. 1995).

1.3 Management And Biological Control Of *A. tsugae*

In Asia and western North America, natural enemies and host resistance likely regulate *A. tsugae* populations (McClure 1995, Sasaji and McClure 1997, McClure et al. 1999, Montgomery et al. 1999, Montgomery et al. 2002), illustrated in part by planted ornamental eastern hemlock trees flourishing in Seattle, WA and Osaka Japan where *A. tsugae* are native (Mausel 2005, Lamb et al. unpublished results). The family Adelgidae generally lacks any known parasitoids (Clausen 1978). Researchers have focused on searching for effective predators (Montgomery and Lyon 1996), entomopathogenic fungi (Costa et al. 2005), and bacterial endosymbionts (Shields and Hirth 2005). *A. tsugae* seems suitable for biological control because it is sessile and lives on trees for long periods of time. The sessile lifestyle leaves them exposed to predators and pathogenic infection. Successful biological control against pests found within the family Adelgidae has only been successful for the *Pineus* genus (Mills 1990). Classical biological control is a strategy that maintains pest populations below damaging levels by releasing effective self-sustaining natural enemies from the pest's native range (Debach and Rosen 1991). Due to the vast geographic range of native hemlock species in the eastern United States and the high cost of applying effective insecticides; chemical application is only viable in landscape plantings

or for short-term protection of high value trees. Since there are no known parasitoids or species-specific pathogens effective against Adelgidae, efforts to manage *A. tsugae* have focused on establishing a complex of predators to attack each life stage (Montgomery and Lyon 1996, Cheah et al. 2004). Foreign explorations for prey-specific predators of *A. tsugae* in Asia and western North America have been undertaken (McClure 1987, Montgomery and Lyon 1996, Cheah et al. 2004) and several predators have been released. However, establishing a complex of predators can be risky if competition among predators is poorly understood. Predator species may provide a synergistic effect and provide increased pest suppression (Heinz and Nelson 1996, Losey and Denno 1998, Onzo et al. 2004), or they may interact negatively, reducing overall predator efficacy (Rosenhiem 2001, Spiller 1986). Flowers et al. (2005, 2006) evaluated inter- and intraspecific competition among three coleopteran predators of *A. tsugae*: two coccinellids, *Sasajiscymnus tsugae* Sasaji and McClure, *Harmonia axyridis* Pallas, and the derodontid beetle *Laricobius nigrinus* Fender. It was concluded that the incorporation of additional conspecific and heterospecific predators increased the total amount of feeding on *A. tsugae* ovisacs, though it was not possible to determine any synergistic effects. It appeared that the presence of these species together would have minimal negative effects, partly because of their different phenologies.

Some predators having a known association with *A. tsugae* in Asia have been studied. In Japan, a mite *Diapterobates humeralis* Hermann (Oribatida: Ceratozetidae) was found to eat the cottony flocculence and cause adelgid eggs to be exposed to both the environment and predation (McClure 1995). It is a common and effective predator in Japan. It is already present in North

America, where it has low fecundity, is very difficult to rear, and consequently deemed unsuitable as a biological control agent (Cheah and McClure 1996).

Exploration for natural enemies occurred from 1995 to 1997 in the Chinese provinces of Yunnan, Sichauan, and Shannxi (Yu et al. 1997, Wang and Gordon 1998). Researchers collected several species of Coccinellids from the tribe Scymnii, known as predators of homopterous insects (Pang and Gordon 1986). *Scymnus* is the largest genus in Coccinellidae and prefer to feed on adelgids, but will feed on aphids (Montgomery et al 1999, Butin et al. 2002). Three *Scymnus* beetles are of interest to researchers: *Scymnus camptodromus* Yu et Liu (Coleoptera: Coccinellidae), *Scymnus ningshanensis* Yu et Yao (Coleoptera: Coccinellidae), and *Scymnus sinuanodulus* Yu et Yao (Coleopera: Coccinellidae). *S. sinuanodulus* was released in the Appalachian mountains of Georgia (Asaro et al. 2005) and *S. ningshanensis* was released in Massachusetts in 2007 (Montgomery et al. 2007). Sleeve cage experiments conducted by Butin et al. (2003) found that both species significantly reduced *A. tsugae* populations. Release of *S. camptodromus* awaits development of adequate rearing techniques (Montgomery et al. 2007). Studies evaluating the impact of released *Scymnus* beetles are currently underway.

Field releases of a multivoltine coccinellid predator from Japan, *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae) (Sasaji and McClure 1997) for control of *A. tsugae* began in 1995 with over a million beetles released at hundreds of sites in 15 eastern states by 2004 (Cheah et al. 2004). Since then, this number has risen to > 3 million beetles (Onken and Keena 2008). Phenology of predator and prey are synchronized (Cheah and McClure 2000). Adults of *S. tsugae* emerge in late March to lay eggs on the *A. tsugae* sistentes generation (Cheah and

McClure 1996). Although Cheah (2008) indicated that in the majority of sites where *S. tsugae* has been released, HWA levels have been reduced from pre-release levels, there is no hard data to show that this predator is having any type of measurable impact on HWA after 15 years of releases.

Tetraphleps galchanoides Ghauri (Hemiptera: Anthocoridae) exists in Sichuan and Yunnan provinces in China, and is an avid predator of *A. tsugae*. *T. galchanoides* is undergoing host-range testing and is another potential addition to the biological control program (McAvoy et al. 2007). The Anthocoridae family is the only hemipteran group that has been considered for biological control of adelgids (Kohler et al. 2008). *Tetraphleps raoi* Ghauri, native to Pakistan, was responsible for the control of *Pineus pini* (Macquart) in Kenya (Aloo and Karanja 1986, Mills 1990, Zilahi-Balogh et al. 2002).

A survey conducted in the American Pacific Northwest for predators and parasitoids of *A. tsugae* (Kohler et al. 2008) showed that the majority of species collected (77.2%) were from the orders Coleoptera or Diptera. After two years of rearing out potential predators, the authors found that the collected dipterans were more susceptible to parasitism than the collected coleopterans. Since Diptera are more susceptible to parasitism their use as biological control agents may be limited; however, Kohler et al. (2008) did discover two species that may have potential as biological control agents; *Leucopis argenticollis* Zetterstedt (Diptera: Chaemyiidae) and *Leucopis atrifacies*. Both species had low parasitization rates that did not exceed 23%. *L. atrifacies* may prove to be a good addition to the biological control complex against *A. tsugae*

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but as *L. argenticollis* already exists in the eastern United States (McAlpine and Tanasijtshunk 1972) it should not be introduced from the Pacific Northwest.

Between 2001 and 2003 field studies were conducted to assess the efficacy of several fungal strains collected from infected hemlocks throughout the western United States and Asia (Reid et al. 2002). Attempts on manufacturing a formulation to effectively carry fungal spores into the environment to infect *A. tsugae* are being conducted (Skinner et al. 2003). Research is needed to determine if fungal spores impact predators already released into hemlock stands.

Research at Virginia Tech. has focused on several species of *Laricobius* found in the family Derodontidae. This small family is known as the tooth-necked fungus beetles because of the flattened lateral margins of the pronotum (Downie and Arnett 1996). Four genera occur in this family: three are mycophagous and one, *Laricobius* Rosenhauer, is predaceous on adelgids (Lawrence and Hlavac 1979, Lawrence 1989, Leschen 2000). All species in the genus *Laricobius* prey on Adelgidae, which feed only on Pinaceae (Lawrence and Hlavac 1979, Havill and Footitit 2007, Zilahi-Balogh, 2001). The *Laricobius* species being examined for biological control of *A. tsugae* are *Laricobius nigrinus* Fender, *Laricobius rubidus* LeConte, and *Laricobius osakensis* Montgomery and Shiyaki (proposed).

1.4 *Laricobius* Predators

Laricobius nigrinus Fender (Coleoptera: Derodontidae) is native to the western United States and is a prey-specific predator of *A. tsugae* (Zilahi-Balogh et al. 2002). First imported from Victoria, British Columbia Canada, into Virginia in 1997 for host range testing (Zilahi-Balogh et al. 2002, 2003), Virginia Tech began mass rearing and releasing *L. nigrinus* into many eastern states as part of a biological control program. Since 2003 *L. nigrinus* has been released and is established in 60% of the hemlock stands where it has been released and monitored at the outset of the effort (Mausel et al. 2010). The predator is univoltine and has phenological synchrony with *A. tsugae*. Both the predator and prey are active in the fall, winter, spring, and aestivate in the summer (Zilahi-Balogh et al. 2003). Adults emerge from the soil in the fall, disperse to hemlock branches, feed on *A. tsugae*, oviposit, and then die. It is possible that *L. nigrinus* use host volatiles in host searching behavior (Franz 1958, Broeckling and Salom 2002) but further research is needed to confirm this. Lamb et al. (2005a) showed that the predator survived and reproduced successfully in southwest Virginia from November to April in temporary branch enclosures. *L. nigrinus* females synchronize oviposition with sistentes generation production of progredientes eggs (Zilahi-Balogh et al. 2003). The larvae are predaceous and consume *A. tsugae* eggs, but are considered inefficient feeders because they leave behind uneaten eggs. This behavior may enhance the mortality of *A. tsugae* because the uneaten eggs become loose and wind or rain may dislodge them (McClure 1995). *L. nigrinus* is relatively free of natural enemies in the eastern United States. It is possible that generalists may occasionally prey on *L. nigrinus,* however, previously released *A. tsugae* predators do not appear to drastically compete or prey on them (Flowers et al. 2005, 2006).

Laricobius rubidus LeConte (Coleoptera: Derodontidae) is the only native *Laricobius* in eastern North America. It has a geographic range from North Carolina to New Brunswick and west to Michigan (Clark and Brown 1960, Lawrence 1989). *L. rubidus* feeds exclusively on adelgids and can complete development on a diet of *A. tsugae* (Zilahi-Balogh et al. 2005). The primary host of *L. rubidus* is the pine bark adelgid, *Pineus strobi* Hartig (Hemiptera: Adelgidae) on white pine, *Pinus strobus* L. (Clark and Brown 1960). More recently *L. rubidus* has been found associated with *A. tsugae* infested hemlock (Montgomery and Lyon 1996, Wallace and Hain 2000). *L. rubidus* has a lifecycle more synchronized with its primary prey and includes a hibernal and aestival diapause (Montgomery and Lyon 1996). However, Mausel et al. (2008) documented the ability of *L. rubidus* to survive and feed on *A. tsugae* during the winter in southwestern Virginia, suggesting the insect goes through quiescence instead of diapause during the winter. Although its life cycle is not as well synchronized with *A. tsugae* as is *L. nigrinus,* adults and larvae are present when suitable *A. tsugae* life stages are present. In time, it is possible that *L. rubidus* may become a predator of consequence where white pines and hemlocks grow together or near each other.

Laricobius osakensis Montgomery and Shiyaki (proposed) (Coleoptera: Derodontidae) is a third and more recently discovered species from Japan that has been under study in the Virginia Tech Beneficial Insect Quarantine Laboratory since 2006. *L. osakensis* is a prey-specific predator of *A. tsugae* and initial observations indicate that it may have a more voracious appetite than *L. nigrinus* (Salom and Lamb 2009). Larvae of *L. osakensis* are not partial feeders of *A. tsugae* ovisacs (personal observation) and this behavior may result in higher mortality of *A. tsugae. L. osakensis* is univoltine, phenologically synchronized with *A. tsugae* (Salom and Lamb 2009),

and may become the next addition to the complex of predators for hemlock woolly adelgid control.

1.5 Establishing Effective Biological Control Programs

The negative effects on biodiversity caused by hemlock decline are extensive (Yamasaki et al. 1999; Brooks 2001; Evans 2002; Snyder et al. 2002; Ross et al. 2003, 2004; Buck et al. 2005; Ellison et al. 2005; Lishawa et al. 2007). Hemlock mortality changes ecosystem processes, structure and water quality (Jenkins et al. 1999; Stadler et al. 2005, 2006; Cobb et al. 2006; Yorks et al. 2003), and an effective biological control program is essential to protect our hemlock ecosystems. Successful biological control programs are difficult to establish (Hall and Ehler 1979, Williamson 1996). Problems associated with implementing successful programs include poor synchrony, adaptability, climate matching, dispersal, geographical race, or the poor searching abilities of predators (Lockett and Palmer 2003). Biological control programs with an optimal release strategy (Campbell 1976, Center et al. 2000, Clark et al. 2001) and a plan for long-term sampling (Soper et al. 1988, Humble 1994, Mo et al. 2000) may have increased chances of success.

Success of the introduction of a natural enemy for biological control of an invasive pest is to some extent under the control of the experimenter and is dependent on factors that affect establishment (Hopper and Roush 1993, Stron and Croft 1996, Daane and Yokota 1997, Dray et al. 2001). A successful biological control agent of *A. tsugae* must be able to establish and populate quickly to effectively reduce prey populations before irreversible damage occurs to trees. Irreversible damage can occur rapidly in old growth trees because they are less resilient to

pest damage. Understanding how many individuals to release for establishment to be effective is one important issue when establishing biological control programs (Shea and Possingham 2000, Clark et al. 2001, Grundy and Maelzer 2002, Jung et al. 2004, Mausel et al. 2010). Other important factors affecting success include release frequency, time of release, release stage of biological control agent, release site characteristics, pre-release acclimation, release equipment, pest density, weather, alternate hosts, natural enemies, dispersal, genetics, and detection efforts (Center et al. 2000, Clark et al. 2001, Damon and Valle 2002, Grundy and Maelzer 2002, Norris et al. 2002). One important factor we need to understand is the potential competitive interactions that may occur among natural enemies.

An understanding of the competitive interactions among the three *Laricobius* species is essential to evaluate the prospects for a successful biological control program for HWA. Interactions among predators may impact their efficacy and should be understood before releasing additional *Laricobius* species. In general, 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). 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). Competitive interactions are usually directed toward species with the greatest temporal or spatial seasonal overlap (Wilson 1971). Although more difficult to detect, indirect competition among species may also occur, where predator activity or behavior is negatively affected as the result of cues, often chemical or tactile, from other predators. These interactions can change community structure favoring 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. All three *Laricobius* species (*L. rubidus, L. nigrinus, L. osakensis*) under investigation for biological control of HWA share spatial and temporal overlap and are prey-specific on the same target prey. The endemic *L. rubidus* was previously thought to undergo a hibernal diapause; however, the combination of having an abundant alternate host coupled with warmer temperatures may allow *L. rubidus* to become more synchronized with both *A. tsugae* and other *Laricobius* predators (Mausel et al. 2008). *L. nigrinus* has become established, and *L.* osakensis has been petitioned for release. Mating attempts have been observed in the laboratory among all three species, suggesting that these species are not antagonistic toward one another. Flowers et al. (2005, 2006), showed that three unrelated predator species of *A. tsugae*, one a generalist and the other two specialists, had minimal impact on each other. *L. nigirinus* showed more intraspecific than interspecific competition. Before *L. osakensis* is released, it would be prudent to determine the potential interactions among these three *Laricobius* species under laboratory conditions. This information is helpful in optimizing field release strategies and will help address an overall goal of assessing the compatibility of these 3 *Laricobius* spp. with respect to HWA biological control.

Research Objectives:

The underlying hypothesis is that *Laricobius rubidus, L. nigrinus* and *L. osakensis* are compatible and can co-exist in the same ecosystem. Specific experiments were conducted to evaluate:

1. Feeding, egg production and survivorship of individual adult female *L. nigrinus, L. rubidus* and *L. osakensis* under simulated natural conditions in the laboratory.

2. Adult predator feeding on predator eggs when offered two different prey-densities under simulated natural conditions in the laboratory.

3. Feeding, egg production and survivorship of conspecific and congeneric groups of adult *Laricobius* predators, to determine if any inter- or intra-specific interactions occur when adults are in groups under simulated natural conditions in the laboratory.

4. Feeding and survivorship of *Laricobius* species larvae when placed in conspecific and congeneric groups under simulated natural conditions in the laboratory, and determine if any inter- or intra-specific interactions occur among groups of larvae.

5. Feeding, egg production and survivorship of adult *Laricobius* predators in conspecific or congeneric groupings within branch enclosed field sleeve cages.

Chapter 2

Assessing Performance And Compatibility Of Three *Laricobius* Species, Predators Of *A. tsugae,* In Laboratory Assays

Abstract

There is potential for three *Laricobius* species predators (Coleoptera: Derodontidae) to simultaneously inhabit HWA-infested hemlock stands in the eastern U.S. through direct introduction of two non-native species and indirect ingression by a native species. The competitive interactions among three species of *Laricobius* (*Laricobius nigrinus* Fender*, Laricobius rubidus* LeConte*, Laricobius osakensis* Montgomery & Shiyaki proposed), feeding on hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae)*,* were investigated in the laboratory. In individual adult assays all three species oviposited in *A. tsugae* ovisacs; however *L. rubidus* oviposited significantly fewer eggs than either *L. nigrinus* or *L. osakensis*. Predator survival was high and predation on ovisacs did not differ among species. When *Laricobius* adults were placed in congeneric or conspecific groups of 3 beetles per assay, fewer eggs were again laid by *L. rubidus*. Egg production by the congeneric group did not differ from *L. osakensis* and *L. nigrinus* conspecific groups. Adult predation differed significantly when placed in congeneric or conspecific groups. Conspecific groups of *L. osakensis* adults and larvae fed on more HWA than either *L. nigrinus* or *L. rubidus* conspecific groups and the congeneric group of all 3 species. No significant differences among groups of adults and larvae in survivorship were observed. Predation on predator eggs by adult *L. nigrinus* and *L. osakensis* did not differ significantly by egg species or prey-density. Predation averaged < 1 predator egg over 6 days for all species. The findings indicate that survival, egg production, and predation of the three *Laricobius* species were not negatively impacted by the presence of either conspecifics

or congenerics, suggesting that these species may be compatible for use in biological control of *A. tsugae*

1. Introduction

Hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a major pest of eastern hemlock, *Tsuga canadensis* L. Carriere, and Carolina hemlock, *Tsuga caroliniana* Engelmann, in the eastern U.S. *A. tsugae* in the eastern United States is believed to have originated from Japan where it poses no threat to native *Tsuga* species (Havill et al. 2006). It was first discovered in 1951 near Richmond, VA. and has spread throughout a large portion of the hemlock range causing widespread mortality in the eastern United States (Cheah et al. 2004). Hemlock in the eastern U.S. plays many important roles, both environmentally and commercially. It is a dominant climax species occupying 7.7 million ha of forested land (Schmidt and McWilliams 1996), is shade tolerant, and creates unique microclimates that support many terrestrial and aquatic species (Evans et al. 1996, Ward et al. 2004). Hemlocks comprise 22 percent of the total volume of softwood grown in the northeastern U.S. Many hemlock cultivars are often planted in ornamental settings where tree mortality may detract from private and public property values (Ward et al. 2004, Battles et al. 1999). As *A. tsugae* began spreading throughout the native hemlock range in the eastern U.S., the economic and environmental impacts became apparent. Due to the damage inflicted upon the native hemlock species by *A. tsugae* in the eastern U.S. and the lack of effective management strategies, research has focused on a diverse array of activities with a long-term goal of protecting and preserving these two species. Activities include attempts to develop resistant eastern hemlock hybrids (Lagalante and Montgomery 2003, Bentz et al. 2005, Montgomery et al. 2005, Playfoot and Ward 2005), conservation of germplasm (Tighe et al. 2005), and chemical control with systemic

insecticides (McClure 1991, 1995, Cowles et al. 2006). Host-resistance research focuses more on recovery after outbreaks have damaged the stands and chemical control when used alone is a short-term location-specific management option.

For long-term sustainable management of HWA, research has focused on classical (introduction of a predator complex) and augmentative, (in this case the use of biopesticides), biological control (Cheah et al. 2004). Efforts toward establishing a complex of predators can be risky if interspecific or intraguild competition among predators is poorly understood. Predator species may provide a synergistic effect and increase pest suppression (Heinz and Nelson 1996, Losey and Denno 1998, Onzo et al. 2004), or they may interact negatively, reducing overall predator efficacy (Rosenhiem 2001, Spiller 1986). Flowers et al. (2005, 2006) evaluated inter- and intraspecific competition among three predators of *A. tsugae*: two coccinellids, *Sasajiscymnus tsugae* Sasaji and McClure*, Harmonia axyridis* Pallas*,* and the Derodontid beetle *Laricobius nigrinus* Fender.   It was concluded that the incorporation of additional conspecific and heterospecific predators increased the total amount of feeding on *A. tsugae* ovisacs, though it was not possible to determine any synergistic effects. It appeared that the presence of these species together would have minimal negative effects, partly because of their different phenologies.

More recently, interest has shifted to several species of *Laricobius.* All species in the genus *Laricobius* Rosenhauer (Coleoptera: Derodontidae) prey on Adelgidae, which feed only on Pinaceae (Lawrence and Hlavac 1979, Havill and Footitit 2007, Zilahi-Balogh et al. 2001). The family Derodontidae is comprised of only four genera (Lawrence and Hlavac 1979). The species

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being studied for potential biological control of *A. tsugae* are *Laricobius nigrinus* Fender*, L. rubidus* LeConte*,* and *L. osakensis* Montgomery and Shiyaki (proposed).

Laricobius nigrinus is native to the western United States and is a prey-specific predator of *A. tsugae* (Zilahi-Balogh et al. 2002). The predator was first imported from Victoria, British Columbia Canada, into Virginia in 1997 for host-range testing (Zilahi-Balogh et al. 2002, 2003). Virginia Tech began mass rearing and releasing *L. nigrinus* into many eastern states as part of a biological control program in 2004 (Cheah et al. 2004). It is univoltine and has phenological synchrony with *A. tsugae* (Zilahi-Balogh et. al 2003). Lamb et al. (2005a) showed that *L. nigrinus* survived and reproduced successfully in southwest Virginia from November to April in temporary branch enclosures. *L. nigrinus* has been released into hemlock stands in the eastern United States since 2003 and is considered established in 60% of the stands where it has been released and monitored from the outset (Mausel et al. 2010).

Laricobius rubidus is the only native *Laricobius* spp. in eastern North America. It has a geographic range from North Carolina to New Brunswick and west to Michigan (Clark and Brown 1960, Lawrence 1989). The predator has a primary host of pine bark adelgid, *Pineus strobi* Hartig (Hemiptera: Adelgidae) and thus is more synchronized with its primary food source (Montgomery and Lyon 1996). Recently *L. rubidus* was found associated with *A. tsugae* infested hemlock (Montgomery and Lyon 1996, Wallace and Hain 2000) and studies have documented its ability to survive and reproduce on *A. tsugae* (Zilahi-Balogh et al. 2005). The life history of *L. rubidus* is similar to *L. nigrinus* except that it may include a hibernal diapause in addition to an aestival diapause (Clark and Brown 1960, Montgomery and Lyon 1996, Zilahi-

Balogh et al. 2005), though recent studies indicate *L. rubidus* is active during the winter in southwest Virginia (Lamb et al. 2005b, Mausel et al. 2008). Adults are active in the fall and spring with larvae developing in the spring. Although its life cycle is not as well synchronized with *A. tsugae* as *L. nigrinus,* adults and larvae are present when suitable *A. tsugae* life stages are present. In time, it is possible that *L. rubidus* may become a predator of consequence where white pines and hemlocks grow together or near each other.

Laricobius osakensis, a third and more recently discovered species from Japan, has been under study in the Virginia Tech Beneficial Insect and Quarantine Laboratory since 2006. *L. osakensis* is a prey-specific predator of *A. tsugae* and initial observations indicate that it may have a more voracious appetite than *L. nigrinus*. It is univoltine and is phenologically synchronized with *A. tsugae* (Salom and Lamb 2009). This species is a potential addition to the complex of predators for HWA control.

An understanding of the competitive interactions among the three *Laricobius* species is essential in evaluating their compatibility as part of a biological control program for HWA. In general, 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). 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). Competitive interactions are usually directed toward species with the greatest temporal or spatial seasonal overlap (Wilson 1971). Although more difficult to detect, indirect competition among species may also occur, where predator activity or behavior is negatively affected as the

result of cues, often chemical or tactile, from other predators. These interactions can change community structure releasing the prey population from predator pressure (Rosenheim et al. 1993). Thus, for biological control programs, 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. *L. nigrinus, L. rubidus,* and *L. osakensis* share spatial and seasonal overlap leading to a greater chance of competition. Mating attempts have been observed among all three species suggesting that these species are not antagonistic toward one another. Therefore, potential interactions among these species merit investigation. Information gained from such studies will help to optimize release strategies, provide some insight on the impacts of interactions among these predators, and the roles they will likely play as biological control agents of *A. tsugae*. The experiments reported here were designed to determine if any competitive interactions among these three species of *Laricobius* occur in a laboratory setting.

2. Materials & Methods
2.1 Beetles

Beetles used in all laboratory experiments were collected from the field using beat sheets in the fall of 2007. *L. rubidus* was collected from white pine, *Pinus strobus L.* stands in Montgomery County, Virginia and Boone County, North Carolina. *L. nigrinus* was collected from western hemlock, *Tsuga heterophylla,* in the greater Seattle, WA area and *L. osakensis* was collected from the Kobe Arboretum near Osaka, Japan.

All predator species were maintained under their normal developmental conditions using methods described by Lamb et al. (2005b). They were kept in 2.2 L plastic containers ventilated

with fine polyester mesh (Sefar™). Each container held no more than 20 adults on 5-7 *T. canadensis* branch clippings heavily infested with HWA. Branches were removed weekly and placed into containers with fresh, infested *T. canadensis* clippings to facilitate larval development. Oviposition tests were conducted to determine the sex of each adult beetle using individual 9 x 2.5 cm polystyrene Petri dishes containing HWA-infested *T. canadensis* branch clippings. One *Laricobius* adult was placed in each oviposition arena and observed for 72 h to determine the presence or absence of eggs. Ovipositing females were then used in adult laboratory assays. Branch clippings were removed from the 2.2 L plastic rearing containers as needed and searched to obtain adult or larval predator life stages for experimental assays. Larvae used in experiments were taken from rearing containers. Larval instars were determined by measuring the head capsule width and body length of each larva (Zilahi-Balogh et al. 2003). The temperature used throughout all laboratory experiments was 12°C, a temperature within the normal activity range for all 3 species (Zilahi-Balogh et al. 2005, Salom and Lamb 2009). Egg laying females of all species were placed into an environmental chamber (Percival-Scientific®) set at 12°C and 12:12 LD for at least 48 h before being used in an experiment. Third instar offspring from field-collected beetles were used in larval experiments. Comparing the mean responses of individual females with respective conspecific or congeneric groups being evaluated allowed us to assess intra- and interspecific interference for each experiment.

2.2 Experimental Design

2.2.1 Individual Adult Assays: Egg Production, Predation, And Predator Survival

All laboratory experiments used 9 x 2.5 cm polystyrene Petri dishes (Fisher Scientific®) lined with moistened filter paper. One adult female *Laricobius* was placed in a Petri dish with clippings of *T. canadensis* infested with 60 *A. tsugae* ovisacs. The Petri dishes were placed into

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an environmental chamber set at 12°C and 12:12 LD for 6 d. Ten replicates of each *Laricobius*

species were used. Egg production and predation were quantified after 6 d by taking counts of

predator eggs produced and number of ovisacs showing evidence of predation, respectively.

Experiments were conducted from late March through April 2009 during which all three species

were producing eggs. *L. rubidus* enters peak egg production slightly later in the year than either

L. osakensis or *L. nigrinus*. *L. rubidus* egg production may have been impacted due to the time

of year these experiments took place. Evidence of predation was based on puncture marks or

signs of feeding on ovisacs. Survival of individual adults was recorded as a binomial response, 1

= alive and 0 = dead.

2.2.2 Conspecific And Congeneric Groupings: Egg Production, Predation, And Predator Survival

Three *Laricobius* adult females of the same species or one adult female of each species (3 total)

were placed in a Petri dish containing 15-20 cm of *T. canadensis* infested with 80 *A. tsugae*

ovisacs. In the larval studies each Petri dish contained 80 *A. tsugae* ovisacs and three 3rd instars

of the same species or one, 3rd instar of each species (3 total). All Petri dishes were placed into

an environmental chamber set at 12°C and 12:12 LD for 6 d. There were ten replicates for each

species or grouping. Each adult female or 3rd instar was marked with a small amount of non-

toxic water based opaque paint (Marvy Opaque Stix®) to indicate species. All experiments were

allowed to continue for 6 d. Egg production was quantified by counting the number of predator

eggs produced by each species. Predation was quantified for adult and larval assays by counting

the number of ovisacs with evidence of predation after 6 d. Survival for both adult and larval

assays was recorded as a binomial response, 1 = alive, 0 = dead for all species within each group.

2.2.3 Predation Of Predator Eggs And *A. tsugae* Ovisacs By Adult *Laricobius* Species At Two Prey-Densities

Choice tests were used to examine predation of predator eggs by *Laricobius* species at high (40 sistentes ovisacs) and low (20 sistentes ovisacs) *A. tsugae* densities, prey densities likely to be found in the field. Ten replicates containing eggs from *L. nigrinus* and *L. osakensis* at both densities were conducted. Five replicates containing eggs from all three species were completed at the low-density food source only, due to a lack of available *L. rubidus* eggs. Experiments containing *L. rubidus* were analyzed separately. Replicates containing only *L. nigrinus* and *L. osakensis* eggs were carried out in Petri dishes holding two small branch clippings of *T. canadensis*, each infested with half the total number of *A. tsugae* ovisacs, together with two eggs from each species (4 eggs total), and one adult female *Laricobius*. Two eggs from each *Laricobius* species were placed singly in sistentes ovisacs on a branch clipping, at the typical oviposition site of the *Laricobius* species (Zilahi-Balogh et al. 2003) with a fine-tip brush. A total of four predator eggs (2 conspecific eggs per *T. canadensis* clipping x 2) were placed in each Petri dish. Each *T. canadensis* clipping was marked with a small piece of differently colored tape indicating egg species for all experiments. Replicates containing all predator egg species including *L. rubidus* eggs were carried out in Petri dishes holding three small branch clippings of *T. canadensis*, each infested with a third of the total number of *A. tsugae* ovisacs, two eggs from each species (6 eggs total) and one adult female *Laricobius*. These replicates included a total of six predator eggs (2 conspecific eggs per *T. canadensis* clipping x 3) placed in each Petri dish and each *T. canadensis* clipping was marked with a small piece of differently colored tape indicating egg species for all experiments. One adult female *Laricobius* species was released into the center of the Petri dish at an equal distance from all *T. canadensis* clippings.

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The number of conspecific or congeneric predator eggs consumed by each species, predator eggs produced, sistentes ovisacs preyed upon, and survival were counted after five days. Predator survival was recorded as a binomial response 1 = live and 0 = dead.

2.3 Statistical Analysis

A general linear model with type III fixed effects was used to analyze data. Tukey's honestly significant difference test was used to separate means $p < 0.05$. Species combinations and *A. tsugae* densities were independent variables. Eggs produced, ovisacs preyed upon, and predator eggs consumed were dependent variables. Survival was recorded as a binomial response and analyzed using PROC GLIMMIX (SAS Institute 2009), a generalized linear mixed model used to analyze count data with binomial or Poisson distributions (SAS Institute 2009). Survival data were transformed to achieve normality and equality of variances using the Newton-Raphson method, and means were adjusted for multiple comparisons using Bonferroni $p < 0.05$.

3. Results

3.1 Individual Adult Assays: Egg Production, Predation, And Predator Survival

Significant differences were found in egg production among the three *Laricobius* species (F = 7.75; df = 2,21; p = 0.01). *L. rubidus* produced fewer eggs over 6 d than *L. nigrinus* and *L. osakensis* (Table 1). Predation on ovisacs was not significantly different among species (F = 0.96; df = 2,21; p = 0.40), ranging from 3 to nearly 4 ovisacs preyed upon per day. Predator survival was high overall, with no significant difference among species (F = 0.58; df = 2,27; p = 0.56).

Table 1. Mean number of predator eggs produced ± S.E., ovisacs preyed upon ± S.E., and percent survival of individual adult female *Laricobius* after 6 d in laboratory assays.

Species	N	Mean Eggs Produced ± S.E.	Mean Ovisacs Preyed Upon ± S. E.	% Survivorship
L. nigrinus	7	6.8 ± 2.5 a[1]	19.2 ± 3.5 a	70 a
L. osakensis	9	11.0 ± 3.6 a	23.0 ± 1.7 a	90 a
L. rubidus	8	0.8 ± 0.3 b	18.0 ± 3.0 a	80 a

[1]Means within columns followed by different letters are significantly different (Tukey's HSD test; $p < 0.05$).

3.2 Conspecific And Congeneric Groupings: Egg Production, Predation, And Predator Survival

Significant differences were found in egg production among *Laricobius* species when adults were placed in conspecific or congeneric groupings (F = 6.36; df = 3,32; p = 0.0017). *L. rubidus* produced fewer eggs than the other species alone and grouped together (Table 2). *L. nigrinus* and *L. osakensis* produced an average of 1.3 and 1.4 eggs/day/female, respectively, compared with only 0.6 eggs/day/female *L. rubidus.* The congeneric groups averaged 1.5 eggs/day/female. Egg production may have differed among adult predators, as eggs from all species are impossible to differentiate, for comparative purposes data broken down in eggs/day/female is based on pooled data.

Net predation on *A. tsugae* ovisacs differed significantly among adult predator combinations (F = 9.30; df = 3,32; p = 0.001) (Table 2). *L. osakensis* fed on more ovisacs than *L. nigrinus* or *L. rubidus.* The number preyed upon by combining the three species did not differ significantly from *L. osakensis* or *L. nigrinus.* Predation by *L. rubidus* was the lowest among all treatments. Similar to the individual assays, there were no significant differences among groups of adults in survivorship (F = 2.20; df = 3,38; p = 0.10).

Table 2. Mean number of predator eggs produced ± S.E., ovisacs preyed upon ± S.E., and percent survival of adult *Laricobius* in conspecific or congeneric groups after 6 d in laboratory assays.

Species	N	Mean Eggs Produced ± S. E.	Mean Ovisacs Preyed Upon ± S. E.	% Survivorship
3 *L. nigrinus*	10	24.0 ± 1.6 a[1]	35.0 ± 2.5 b	100 a
3 *L. osakensis*	9	26.0 ± 4.0 a	45.2 ± 2.6 a	80 a
3 *L. rubidus*	7	11.3 ± 2.5 b	27.0 ± 2.0 c	70 a
1 per species	10	27.0 ± 2.3 a	39.0 ± 2.0 ab	83 a

[1]Means within columns followed by different letters are significantly different (Tukey's HSD test; $p < 0.05$).

Net predation differed significantly among groups of *Laricobius* larvae (F = 5.05; df = 3, 19; p = 0.0097). Conspecific groups of *L. osakensis* larvae had relatively higher net predation, but it was not statistically different from *L. nigrinus* (Table 3). Predation by the combined species treatment and *L. rubidus* was significantly less (about half), compared with predation by *L. osakensis*. The low net predation of the combined species treatment may be attributed to the fact *L. osakensis* in the combined treatment died during the experiment due to accidental use of toxic paint. All *L. osakensis* larvae in each replicate of the combined treatment died within the first 12 h from the start of the bioassays. Survivorship between *L. nigrinus* and *L. rubidus* larvae not marked with toxic paint was high (50-77%), and no significant differences in survivorship between larvae of the two species still alive were found (F = 0.87; df = 3,19; p = 0.47).

Table 3. **Mean number of ovisacs preyed upon ± S.E. and percent survivorship of** *Laricobius* **larvae in conspecific or congeneric groups after 6 d in laboratory assays.**

Larvae Species	N	Mean Ovisacs Preyed Upon ± S. E.	% Survivorship
3 *L. nigrinus*	7	37.0 ± 6.0 ab[1]	71 a
3 *L. osakensis*	6	61.0 ± 6.5 a	77 a
3 *L. rubidus*	4	26.0 ± 8.0 b	50 a
1 *L. nigrinus* & 1 *L. osakensis*[2]	6	32.3 ± 6.5 b	66 a

[1]Means within columns followed by different letters are significantly different (Tukey's HSD test; $p < 0.05$).

[2]*L. osakensis* died within the first 12 h of the experiment (in all congeneric groups) and was excluded

3.3 Predation Of Predator Eggs And Sistentes Ovisacs By Adult *Laricobius* Species At Two Prey-Densities

The model did not identify a significant interaction (F = 0.83; df = 1,6; p = 0.36) as a result data were pooled for species. Predation on eggs by adult *L. nigrinus* and *L. osakensis* species did not differ significantly by egg species (F = 0.03; df = 1,6; p = 0.85) or prey-density (F = 2.68; df = 1,6; p = 0.11) (Table 4). *L. osakensis* fed on an average of 0.50 predator eggs compared with 0.17 eggs by *L. nigrinus* over 6 d.

When *L. rubidus* eggs were presented to adult *Laricobius* species in the presence of low prey-density, no significant differences in egg predation were found (F = 0.82; df = 2,12; p = 0.46) (Table 5). Values for all species were < 1.

Additional results found the count of HWA ovisacs consumed did not differ significantly by *Laricobius* adults (F = 0.16; df = 2,3; p = 0.85) (no table); however, net predation by both *L. nigrinus* and *L. osakensis* increased by almost 50 percent when higher numbers of ovisacs were available (F = 17.80; df = 1,3; p = 0.0001). The mean number of ovisacs showing evidence of predation at low food density (20 ovisacs) was 6.27 over 6 d, while the mean number of ovisacs showing evidence of predation at high food density (40 ovisacs) was 10.74 over 6 d. Survivorship was high and did not differ significantly for any species (F = 0.51; df = 2,47; p = 0.60).

Table 4. Mean number of predator eggs consumed ± S.E. by adult *Laricobius* at high (40 ovisacs) and low (20 ovisacs) prey densities after 6 d in laboratory assays.

Adult *Laricobius* spp.	Species eggs consumed	N	Mean # *Laricobius* eggs consumed ± SE
High Prey Density			
L. nigrinus	**L. nigrinus**	9	0.11 ± 0.11 a[1]
L. osakensis		9	0.11 ± 0.11 a
L. nigrinus	**L. osakensis**	9	0.22 ± 0.22 a
L. osakensis		9	0.44 ± 0.24 a
Low Prey Density			
L. nigrinus	**L. nigrinus**	9	0.22 ± 0.22 a
L. osakensis		9	0.33 ± 0.23 a
L. nigrinus	**L. osakensis**	9	0.78 ± 0.28 a
L. osakensis		9	0.55 ± 0.24 a

[1]Means within columns (for each prey density) followed by different letters are significantly different (Tukey's HSD test; $p < 0.05$).

Table 5. Mean number of *L. rubidus* eggs consumed ± S.E. by adult *Laricobius* when offered a low (20 ovisacs) prey density after 6d in laboratory assays.

Adult *Laricobius* spp	N	Mean # of *L. rubidus* eggs consumed ± S.E.
L. nigrinus	5	0.20 ± 0.20 a[1]
L. osakensis	5	0.80 ± 0.40 a
L. rubidus	5	0.80 ± 0.40 a

[1]Means within columns followed by different letters are significantly different (Tukey's HSD test; p < 0.05).

4. Discussion

All adult *Laricobius* species oviposited in *A. tsugae* ovisacs, but fewer eggs were oviposited by *L. rubidus* than *L. nigrinus* or *L. osakensis*. In contrast, predation rates over 6 d were similar among all three species as adults in individual experiments (Table 1). This suggests that at 12° C all three species are active. *L. rubidus* is generally active from March to June (Zilahi-Balogh et al. 2005), *L. nigrinus* (Zilahi-Balogh et al. 2003) and *L. osakensis* (Salom and Lamb 2009) from October to June. The latter two species are active earlier and produce more eggs overall when compared with *L. rubidus*. Although *L. rubidus* feeds and reproduces on *A. tsugae,* its overall impact on *A. tsugae* is much less than that of the other two species because it produces few eggs.

When adult predators were placed in conspecific and congeneric groups, conspecific groupings of *L. osakensis* preyed on more ovisacs than conspecific groups containing either *L. nigrinus* or *L. rubidus* (Table 2). Assays containing congeneric groups exhibited similar net predation when compared with assays containing conspecifics, indicating that the different species were not interfering negatively with each other. Overall, mean net predation was 2x greater in assays containing 3 predators than in assays containing only one predator. It is possible that the amount or quality of prey, or the assay itself limited the ability of the three species to work together to achieve and additive predation rate. Egg production was consistently higher in groups of *L. osakensis* and *L. nigrinus* when compared with groups of *L. rubidus* (Table 2). Overall egg production increased in experiments containing three predators compared with experiments containing one predator for both *L. nigrinus* and *L. osakensis.* Survivorship was high for all species, suggesting that experimental conditions were suitable and negative interactions were low (Table 2).

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Laricobius larvae placed in conspecific and congeneric groupings preyed upon similar numbers of ovisacs. Though no significant differences were found in predation among groups of *Laricobius* larvae, groups containing three *L. osakensis* larvae preyed upon a larger number of ovisacs than groups containing *L. nigrinus* or *L. rubidus* (Table 3). *L. osakensis* larvae were observed consuming whole ovisacs, leaving nothing behind while other *Laricobius* predators fed partially on ovisacs. While this seems important, it should be noted that in 1992 a mite, *Diapterobates humeralis* (Hermann) (Oribatida: Ceratozetidae) was studied that did not feed directly on *A. tsugae* eggs but fed on the woolly filaments of the ovisac. This behavior resulted in dislodging *A. tsugae* eggs and accounted for up to 65% egg mortality (McClure 1995). *L. nigrinus* and *L. rubidus* may cause indirect damage to eggs not directly fed upon thereby increasing overall impact. In the experiment with groups containing one of each species, more ovisacs would likely have been fed on if *L. osakensis* had they not died prematurely from paint toxicity. Survivorship was otherwise high suggesting that negative interactions were low among predators. Previous observations of *L. nigrinus* by Lamb et al. (2005a) indicated that larval cannibalism occurs, however, prey-densities were high during the experiment and may have limited interference among predators.

Flowers et al. (2005, 2006) found an inverse relationship in the lab and field between predator egg consumption and *A. tsugae* density that likely arose from a decline in the probability of predators encountering conspecific or congeneric eggs as *A. tsugae* density increased. No inverse relationship between predator egg consumption and prey-density was found in these studies and adult predators consumed both conspecific and congeneric eggs regardless of prey-

density. The absence of egg preference combined with the narrow host range of the predators suggests that predator eggs were preyed upon opportunistically. Eggs of all species are vulnerable to predation and consuming eggs 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). Direct reproductive interference was evident as remnant chorions of predator eggs were found through microscopic examination of branch clippings, indicating cannibalism. Overall, predation by co-existing predator species will have a limited impact on predator egg production through predation of predator eggs (Tables 4, 5). Predation on predator eggs may be greater in these laboratory experiments than in a field environment due to the limited amount of space available for daily activities and the increased probability of finding eggs.

Higher densities of prey led to increased ovisac predation by all species. This density-dependent functional response is consistent with field observations (Chapter 3). Density-dependence is known to be a controlling factor of hemlock woolly adelgid (McClure 1991). Very high populations of hemlock woolly adelgid cause reduced growth of *T. canadensis* (McClure 1991). Growth of *T. canadensis* was reduced once infested with *A. tsugae* causing a reduction in the amount of food available for the adelgid to feed upon, and a subsequent temporary decline in pest populations. Successful biological control agents necessarily respond to changing prey-densities. Experiments containing high and low-prey densities demonstrate that predators will increase or decrease predation based on availability. Lamb et al. (2005a) found predator egg production was also influenced by *A. tsugae* density and predator density. If females were presented with high prey-densities egg production increased. A numerical response was not

found in these laboratory experiments; however, conspecific groups produced fewer eggs per female compared with individual females, probably due to available space. Lack of a numerical response among predators may have been due to the short duration of assays, available space, or prey-density may have been too high, even at the low-density level. Throughout all experiments, adult and larval, predator survival was high. This was due in part to the short duration of experiments, lack of negative interactions and the relatively high amount of prey provided.

Temperature requirements are important to all aspects of insect development and greatly influences egg production (Cheah and McClure 1998, Stathas et al. 2001, Zilahi-Balogh et al. 2002). All laboratory assays were conducted at 12° C, the lower end of the optimal temperature range for *L. nigrinus* and *L. rubidus,* and the upper end of optimal range for *L. osakensis.* Temperature may have influenced both feeding and egg production rates. Other factors that may have influenced the quantity of egg production and predation throughout this study are inadequate prey quality (Palmer and Sheppard 2002), host tree health (Sheppard and Palmer 2004) or nutrient limitation. An additional factor may be the confining limits of the Petri dish as it severely limited predator dispersal or movement to search for more favorable conditions. Although Petri dish assays may not accurately reflect field conditions due to size constraints, the results provide us with guidance about what to expect in the field.

Based on laboratory results in this study, the three *Laricobius* species appear compatible in terms of predation of target prey, oviposition in prey ovisacs, and limited predation on each other. Releasing more than one predator species into the environment should not cause harmful interactions in terms of predation, oviposition, and survival rates. Mating attempts have been observed among these predator species, indicating a non-antagonistic relationship. However, the

result of interbreeding has not yet been examined. It is unknown if mating attempts are successful or if hybrids produced will be reproductively functional. Research into this area is currently under way.

Chapter 3

Performance And Compatibility Of Adelgid Predators, *Laricobius nigrinus* Fender And *Laricobius rubidus* LeConte (Coleoptera: Derodontidae), In Field Sleeve Cage Evaluations

Abstract

Predation, egg production, and survivorship were investigated for *Laricobius nigrinus* Fender and *L. rubidus* LeConte (Coleoptera: Derodontidae), in conspecific and congeneric groups at two prey-densities. Predators were enclosed in sleeve cages on hemlock branches with either a high (>120 ovisacs) or low (<90 ovisacs) *Adelges tsugae* Annand (Hemiptera: Adelgidae) density, in either a congeneric or conspecific grouping for 1 wk in natural hemlock stands located in southwest Virginia and southeast Kentucky in 2009. Experiments were replicated twice during the pre-oviposition stage and twice during the oviposition stage of *A. tsugae*. All branches with caged beetles had greater numbers of ovisacs with evidence of predation than branches caged without beetles. Egg production among predators did not differ at either prey-density; however, experiments demonstrated strong phenological synchrony between *Laricobius* predators and their food source *A. tsugae,* as the predators did not begin producing eggs until their prey did. No differences were found between predators or predator groupings in survivorship, which was high throughout the experiments. Results from this field study support those of the laboratory studies reported in Chapter 2, suggesting that these species can co-exist and will not inhibit each other when found together in hemlock stands infested with *A. tsugae*.

1. Introduction

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is an invasive pest of eastern hemlock, *Tsuga canadensis* L. Carriere, and Carolina hemlock, *T. caroliniana* Engelmann, in the eastern U.S. It is believed to have originated from Japan where it poses no threat to native *Tsuga* species (Havill et al. 2006). First discovered in 1951 near Richmond, Virginia, it has since spread throughout a large portion of the hemlock range causing widespread mortality in the eastern U.S. (Cheah et al. 2004). Eastern hemlocks, *T. canadensis* and *T. carolinia,* are damaged by the feeding activities of HWA. Adelgid nymphs penetrate plant tissues with their stylets to feed on the parenchyma cells that serve as nutrient transfer and storage cells in the xylem rays (Young et al. 1995). Tree health deteriorates due the depletion of photosynthates, inhibiting shoot growth, causing bud mortality, twig dieback, foliage discoloration and premature defoliation. HWA will cause physiological damage once 30% of a trees shoots have been infested (Fidgen et al. 2006). When crown transparency reaches 60%, tree mortality begins (Mayer et al. 2002). High populations of HWA reduce new shoot growth during the following growing season. After an initial outbreak, reduced shoot growth is followed by a reduction in pest density because HWA performs poorly on older shoots (McClure 1991). Both infested hemlock trees and HWA populations generally resume growth after pest densities decline, but the infested hemlock trees never fully recover. Some trees may survive for decades in a cycle of decline and recovery while others may die only four years after the initial infestation occurs. Weakened trees are more susceptible to many environmental stressors such as drought, other insect attacks, and disease, all contributing to mortality (McClure 1991, Young et al. 1995).

In Asia and western North America, natural enemies and host resistance likely regulate HWA populations (McClure 1995, Sasaji and McClure 1987, McClure et al. 1999, Montgomery et al. 1999, Montgomery et al. 2002). This is illustrated in part by planted ornamental eastern hemlock trees flourishing in Seattle, WA and Osaka, Japan where HWA is native (Mausel 2005; Lamb et al. unpublished results). Species in the family Adelgidae generally lack any known parasitoids (Clausen 1978). Consequently, identification of potential biological control agents has focused on searching for effective predators (Montgomery and Lyon 1996), entomopathogenic fungi (Costa et al. 2005), and bacterial endosymbionts (Shields and Hirth 2005). HWA is suitable for biological control because it is sessile and lives on trees for long periods of time. The sessile lifestyle exposes them to predators and pathogenic infection. Successful biological control against pests found within the family Adelgidae has previously only been successful for *Pineus* species (Mills 1990).

Foreign explorations for prey-specific predators of *A. tsugae* in Asia and western North America have yielded a number of potential biological control agents (Salom et al. 2001, Cheah et al. 2004, Onken and Keena 2008). Establishing a complex of predators can be risky if competition among predators is poorly understood. Predator species may provide a synergistic effect and provide increased pest suppression (Heinz and Nelson 1996, Losey and Denno 1998, Onzo et al. 2004), or they may interact negatively, reducing overall predator efficacy (Rosenhiem 2001, Spiller 1986).

There has been significant interest in using several *Laricobius* species as primary components of a biological control program for HWA. All species in the genus *Laricobius* Rosenhauer prey on a species within Adelgidae, a family of herbivores found only on Pinaceae (Lawrence and Hlavac 1979, Havill and Footitit 2007, Zilahi-Balogh et al. 2007). *Laricobius* is in the small coleopteran family Derodontidae, composed of only four genera (Lawrence and Hlavac 1979). The species being examined for biological control of *A. tsugae* are *Laricobius nigrinus* Fender *Laricobius rubidus* LeConte and *Laricobius osakensis* Montgomery and Shiyaki (proposed). *L. osakensis* is a more recent discovery from Japan and has been under study in the Virginia Tech Beneficial Insect and Quarantine Laboratory since 2006. This species is a potential addition to the complex of predators for hemlock woolly adelgid control. As it is still undergoing quarantine studies, it has not been included in these field studies.

Laricobius nigrinus native to the western United States is a prey-specific predator of *A. tsugae* (Zilahi-Balogh et al. 2002). Imported from Victoria, British Columbia, Canada, into Virginia in 1997 for host range testing (Zilahi-Balogh et al. 2002, 2003), *L. nigrinus* has since been released into many eastern states for biological control of HWA. It is univoltine and is phenologically synchronized with *A. tsugae* (Zilahi-Balogh et al. 2003). Lamb et al. (2005a) reported that it survived and reproduced successfully in southwest Virginia from November to April in temporary branch enclosures. *L. nigrinus* is established in 60% of the stands where it was released between 2003-05 and since monitored (Mausel et al. 2010).

Laricobius rubidus is native to eastern North America, with a geographic range from North Carolina to New Brunswick and west to Michigan (Clark and Brown 1960, Lawrence 1989). *L.*

rubidus feeds on and is synchronized with pine bark adelgid, *Pineus strobi* Hartig (Hemiptera: Adelgidae)*, (Montgomery and Lyon 1996). Recently *L. rubidus* was found associated with HWA infested hemlock (Montgomery and Lyon 1996, Wallace and Hain 2000) and studies have documented its ability to survive and reproduce on HWA (Zilahi-Balogh et al. 2005). The life history of *L. rubidus* is very similar to *L. nigrinus* except that it was thought to include a hibernal diapause in addition to an aestival diapause (Clark and Brown 1960, Montgomery and Lyon 1996, Zilahi-Balogh et al. 2005, Mausel et al. 2008). Adults are active in the fall and spring with larvae developing in the spring. Recent studies conducted by Mausel et al. (2008) have shown *L. rubidus* are active in the winter in southwestern Virginia. Although not as well synchronized as *L. nigrinus* with HWA, *L. rubidus* adults and larvae are present when suitable HWA life stages are present. In time, it is possible that *L. rubidus* may become a predator of consequence where white pines and hemlocks grow together or near each other.

There is a potential for competition among *Laricobius* species because of the similar spatial, temporal, and behavioral characteristics that have been observed for these species. Therefore, a study of their potential interactions is necessary. Information gained from such an effort will be helpful in optimizing release strategies, determining the impacts these predators are having, and in describing the roles they will likely play as biological control agents of HWA. These field experiments follow up on laboratory experiments discussed in chapter two. In laboratory experiments few negative interactions were found and groups of congeneric species preyed on similar numbers of prey ovisacs when compared with conspecific groups. The purpose of this study is to determine if any competitive interactions between *L. nigrinus* and *L. rubidus* occur in

a field environment, and if so, identify what they are to supplement the laboratory experiments presented in chapter 2.

2. Materials and Methods

2.1 Beetles

Adult *Laricobius* collected from the field with beat sheets during the fall of 2008 were used for all field experiments. *L. rubidus* was collected from white pine, *Pinus strobus L.* stands in Montgomery County, VA and Boone County, NC. *L. nigrinus* was collected from western hemlock, *Tsuga heterophylla,* around the greater Seattle, WA area. Predator species were maintained in the lab under normal developmental conditions using methods described by Lamb et al. (2005b). They were kept in 2.2 L plastic containers ventilated with fine polyester mesh (Sefar™). Each container held no more than 20 adults and 5-7 *T. canadensis* branch clippings heavily infested with HWA. Branch clippings were removed weekly and placed into rearing containers with fresh, infested *T. canadensis* clippings to facilitate larval development. Containers were maintained in an environmental chamber (Percival-Scientific®) at 12°C and 12:12 LD. Adult *Laricobius* collected during the fall of 2008 were of approximately the same age. Individuals from each species were randomly selected from the 2.2 L plastic containers for use in experiments.

2.2 Experimental Design

Predation, egg production, and survivorship of adult *L. nigrinus* and *L. rubidus* were measured, in both conspecific and congeneric groupings on pre-ovipositing and ovipositing stages of *A. tsugae* (sistentes) at low (< 90 ovisacs) and high (>120 ovisacs) densities. Experiments were

conducted in the field during 2009 using sleeve cages described by Flowers et al. (2006). Natural eastern hemlock stands with moderate to high HWA infestations were selected in southwest Virginia and southeast Kentucky as case study sites. HWA overwinters as developing nymphs (pre-oviposition stage) where ovisacs with no eggs are present. In the early spring HWA adults enter an oviposition stage to produce eggs. Whereas *Laricobius* adults survive on HWA nymphs and adults, their larval progeny require HWA eggs (Lamb et al. 2005b). *Laricobius* females are phenologically synchronized with their prey and do not begin depositing eggs in the ovisacs until HWA begins oviposition (Zilahi-Balogh et al. 2003, Mausel et al. 2008). Pre-oviposition HWA experiments were conducted twice at Mountain Lake (Giles Co, VA) (37° 21' 48.29" N, 80° 33' 03.93" W, Elevation 1237 m), once in January (2009) and once in February (2009). Ovipositing HWA experiments were conducted twice in Kentucky Ridge State Forest (Bell Co., KY) (36° 45' 59.66" N, 83° 47' 02.43" W, Elevation 405 m), during April (2009). Kentucky Ridge State Forest was selected to continue experiments due to the rapid decline of hemlock health in the Mountain Lake area. All experiments used unknown sexed pairs of *Laricobius.*

A generalized randomized complete block design blocked by date was repeated on four dates (two at Mountain Lake and two at Kentucky Ridge State Park). On each date, five *T. canadensis* trees with moderate to high HWA densities were randomly selected. Eight branches were selected from each tree that met the following criteria: 1-2 m height above the ground, with a size of 0.3 x 0.6 m, and HWA density of either < 90 ovisacs (low-density treatment level) or > 120 ovisacs (high-density treatment level). All eight branches received one of three predator treatment combinations or a non-predator treatment control. These were superimposed on the

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two HWA density levels. Baseline counts of HWA density on all eight branches of five trees were established before each evaluation by counting the number of sistentes woolly masses. Intact woolly masses, possessing clear honeydew, and with no apparent damage from predators, were considered alive. 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. Each enclosed branch area contained 250-300 cm of infested foliage at either a high or low ovisac density, either a conspecific or congeneric predator treatment or non-predator control. Conspecific groups contained either two *L. nigrinus* or two *L. rubidus* per cage while congeneric groups contained one of each *Laricobius* species in the same cage. Prey densities are very high in natural infestations, and this experimental design represents the conditions likely to occur in the field. Attempts were made to remove all native predators from branches before cage placement by tapping branches. All conspecific or congeneric groups of predators were introduced at the same time into the enclosures and sealed at the base using cinch ties. Caged control branches were evaluated at both low and high ovisac densities to account for mechanical mortality of HWA during transport from field to laboratory and any potential cage effect. Predators remained in sleeve cages for 6 d during each experiment. At the conclusion of each study, branches were cut and returned to the laboratory. The numbers of surviving adult predators on each branch were recorded as binomial responses (alive = 1; dead = 0). Branch clippings were examined microscopically to obtain counts of predator eggs produced and predation on ovisacs. Predation was quantified by counting the number of sistentes ovisacs showing evidence of predation. Evidence of predation is defined by obvious destruction of woolly ovisacs. Damage includes punctures on adults and/or damage to the exoskeleton and can be used to distinguish *Laricobius* predation from other mortality causes.

3. Statistical Analysis

3.1 Predation And Egg Production

Field experiments followed a generalized randomized complete block design blocked by date and a general linear model with type III fixed effects was used to analyze data. Predation and egg production measures were examined separately by species combination, HWA oviposition stage, and date. The general linear model (PROC GLM; SAS Institute, 2009) included predator species combination, HWA oviposition stage, date, and their interactions as independent categorical variables. Data were tested for normality and equality of variance and analyzed using a two-way analysis of variance (Zar 1998). Tukey's honestly significant differences test was used to separate means and determine significant differences at $p < 0.05$.

3.2 Predator Survivorship

Predator survival was recorded for each individual as a binomial response (alive = 1, dead = 0) and was analyzed separately by predator combination, HWA oviposition stage, and date using GLIMMIX (PROC GLIMMIX; SAS Institute, 2009), a generalized linear mixed model used to analyze count data with binomial or Poisson distributions (SAS Institute 2009). Survival data were transformed to achieve normality and equality of variances using the Newton-Raphson method and means were separated using Tukey's honestly significant differences test at $p < 0.05$.

4. Results

4.1 Predation

Significant differences in net predation on prey ovisacs were found among sleeve cage treatments at both low and high prey-densities (Table 6). All caged control branches had significantly less predation than branches containing conspecific groups or congeneric groups of predators. Conspecific groups of *L. rubidus* and *L. nigrinus* were not significantly different from each other. Similarly congeneric groups were not significantly different from conspecific groups of *L. rubidus* or *L. nigrinus*. The model identified two interactions the first is a predator treatment by prey-density interaction (F = 8.19; df = 3,6; p = 0.0001), due to the fact that predation in the control treatment did not increase with increased prey-density while the species treatment data did. Sleeve cages containing high prey-densities had a significantly greater number of ovisacs preyed upon than sleeve cages containing low prey densities (F = 63.59; df = 1,6 p = 0.0001). When control mean values were subtracted at low prey densities, conspecific groups of predators preyed upon an average of 43 ovisacs over 6 d while congeneric groups preyed upon an average of 35 ovisacs. When subtracting control mean values at high prey-densities, conspecific groups of *L. nigrinus* and *L. rubidus* preyed upon 91 and 78 ovisacs over 6d, respectively. Congeneric groups of *Laricobius* predators preyed upon 97 ovisacs over 6 d. A second interaction observed was between prey-density and date (F = 2.97; df = 3,6; p = 0.0343), possibly caused by the variation in overall prey-densities offered to predators at both high- and low-density food sources during each replication of experiments. When means were plotted the overall trend in predation activity remained the same; however, greater numbers of ovisacs were preyed upon in February in both low and high prey-density sleeve cages. This likely caused the observed interaction.

4.2 Predator Egg Production

Egg production did not differ significantly among conspecific and congeneric treatments ($F = 0.25$; df = 2,7; $p = 0.7779$) at either prey-density ($F = 0.11$; df = 1,7; $p = 0.7404$) (Table 6). Date had a significant effect on predator oviposition ($F = 26.86$; df = 3,7; $p = 0.0001$) (Table 7). The date effect illustrates the transition of *Laricobius* from pre-oviposition to oviposition stage. During the first two dates, HWA had not yet matured to lay HWA eggs. By April, HWA adults were ovipositing. Virtually no predator eggs were produced by either *Laricobius* species before HWA eggs were present. Oviposition by the predators was observed only when HWA eggs were present in April.

4.3 Predator Survivorship

Predator survivorship was fairly high throughout all field experiments and ranged from 57% to 75%. (Table 6) No significant differences were found in survivorship among predator treatments ($F = 0.31$; df = 2,107; $p = 0.73$). Survivorship of all predators in January was significantly lower from the other dates ($F = 3.01$; df = 3,107; $p = 0.033$), and was likely caused by very low temperatures.

Table 6. The mean number of ovisacs preyed upon, *Laricobius* eggs deposited ± S.E., and percent survivorship on high (< 120 ovisacs) and low (> 90 ovisacs) prey densities after 1 week in field sleeve cages. Treatment data pooled over four dates.

Treatment	Prey-density	N	Mean # ovisacs preyed upon ± S.E.	N	Mean # eggs deposited ± S.E.	N	% Survivorship
Control	Low	19	25.1 ± 5.1 c[1]	n/a	n/a	n/a	n/a
Mix	Low	20	60.1 ± 4.2 b	20	1.6 ± 0.4 a	48	63 a
L. rubidus	Low	19	68.1 ± 5.1 b	18	1.3 ± 0.5 a	46	57 a
L. nigrinus	Low	20	68.1 ± 6.4 b	20	1.6 ± 0.5 a	48	75 a
Control	High	21	25.5 ± 7.0 c	n/a	n/a	n/a	n/a
Mix	High	20	122.2 ± 6.1 a	20	1.6 ± 0.6 a	48	73 a
L. rubidus	High	21	102.9 ± 11.0 a	20	1.5 ± 1.9 a	48	69 a
L. nigrinus	High	20	116.4 ± 8.8 a	20	2.0 ± 0.6 a	48	71 a

[1]Means within columns followed by different letters are significantly different (Tukey's HSD; test p < 0.05).

Table 7. Mean number *Laricobius* eggs[1] deposited ± S.E. by date after 1 wk in field sleeve cage studies during pre-oviposition and oviposition stages of their prey, *A. tsugae.*

Date	N	Mean # Predator Eggs Deposited ± S.E.
16-Jan-09	30	0.0 ± 0.00 b[2]
2-Feb-09	30	0.06 ± 0.06 b
11-Apr-09	29	3.24 ± 0.53 a
19-Apr-09	29	3.13 ± 0.38 a

[1]Pooled by species treatment

[2]Means within columns followed by different letters are significantly different (Tukey's HSD test; $p < 0.05$)

5. Discussion

Predation on adelgid populations by *Laricobius* throughout the winter and early spring is important because other predators are not active (Cheah and McClure 2000, Montgomery et al., 2002). Flowers et al. (2005, 2006) found that *L. nigrinus* had the greatest impact on HWA in the spring when the combined actions of predator larvae and adults occur. In the current experiments, higher HWA densities were associated with increased predation by both *Laricobius* spp. Predation for each species combination almost doubled with presence of greater prey density, representing a density-dependent functional response consistent with laboratory observations (Chapter 2). Experiments containing high- and low-density food sources demonstrate that predators will prey upon more adelgids when a larger supply of prey is available, an important characteristic for successful biological control agents (Debach and Rosen 1991). The second interaction found by the model was between prey-density and date. This interaction was likely caused by the overall variation in prey offered to predators inside the sleeve cages at each date. Attempts were made to keep the amount of prey offered at both low and high density levels similar during each replication; however, it was nearly impossible to present predators with exact numbers of prey in each enclosure during all four dates.

Mean temperatures were between 5-8°C at the Mountain Lake Biological Station and 12-18°C at Kentucky Ridge State Forest during the experiments. Low temperatures can lower prey quality (Palmer and Sheppard 2002), host tree health (Sheppard and Palmer 2004) or nutrient availability. January temperatures were very low and may have influenced prey quality, activity, and survivorship of predators. Additional factors potentially affecting predators include the short

duration of experiments and the limits sleeve cages place on predator dispersal to search for more favorable conditions especially during cold weather.

In contrast with laboratory experiments egg production did not differ significantly among the predator treatments. Date had a significant effect on oviposition. Predator egg production began during February and continued through April. This was expected as *Laricobius* larvae prey on HWA eggs produced in the spring. Predator egg production was fairly low throughout these experiments but did increase through time. If cage experiments had been put in place in March, it might have helped capture the onset of oviposition for both predator species and their prey. Overall, the experiments do support previous work by Zilhi-Balogh et al. (2003) and Mausel et al. (2008) that phenological synchrony exists among HWA and its *Laricobius* predators.

Even when temperatures were coldest in February, a high proportion of *Laricobius* predators survived and no significant differences occurred among predator combinations. Negative interactions among congeneric and conspecific groups were not observed. The data reported here provide insight into the presence/absence of inter- specific and intra-specific competition. While these results do not provide definitive conclusions as to the level of competition that may occur under different ecological conditions or longer durations, it can provide guidance regarding the likelihood of potential problems in terms of their predation, egg production, and survivorship. Branch enclosure experiments do not reflect the potential for predator immigration and emigration and the level of competition among predators may have been constrained by the low temperatures experienced. *Laricobius nigrinus* and *L. rubidus* appear to be capable of co-

existing, and are compatible from the perspective of predation of prey and oviposition of progeny.

Chapter 4

4.1 Summary And Conclusions

All three *Laricobius* species appear compatible in regards to predation, egg production and survivorship from the laboratory studies (Chapter 2). Lack of negative interactions between *L. nigrinus* and *L. rubidus* from the field study (Chapter 3) supports the laboratory studies. Based on these results, I believe that the presence of *L. nigrinus* and *L. osakensis* these two in the same ecosystem should not negatively affect their establishment and impact on the target prey. Mating attempts have been observed among these predator species, indicating a non-antagonistic relationship; however, the result of interbreeding has not been examined. As interactions among predators may either directly or indirectly affect biological control, comparison of the responses of conspecific groups and congeneric groupings provided some insight into the presence/absence of inter-specific and intra-specific competition between these two *Laricobius* species. Similar responses from conspecific pairs and congeneric pairs were observed indicating a lack of competition. These results do not provide definitive conclusions as to the level of competition that may occur under different ecological conditions or longer durations. Branch enclosure experiments do not reflect the potential for predator immigration and emigration and the level of competition among predators may have been constrained by the low temperatures experienced.

4.2 Further Study

Additional research that would complement the studies presented here includes the evaluation of potential hybridization among the three *Laricobius* species. The inter- and intra-specific interactions when *L. osakensis* is included in sleeve cages will provide a better understanding of what to expect in the future when this species is included in a biological control program. Further larval studies that include all three species would be helpful to determine if competition occurs among larval stages. Field studies including larval forms of all predators would help determine impact on HWA populations.

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