Assessing Performance and Competition Among Three Laricobius (Coleoptera: Derodontidae) Species, Predators of Hemlock Woolly Adelgid, Adelges tsugae (Hemiptera: Adelgidae)

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ABSTRACT Predation, egg production, and survivorship of Laricobius nigrinus Fender, L. rubidus LeConte, and L. osakensis Montgomery and Shiayki, predators of hemlock woolly adelgid, Adelges tsugae Annand, were investigated in the laboratory and in the field. In individual assays in the laboratory, 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. The number of ovisacs preyed upon was not different between treatments with all three species and those with only L. osakensis or L. nigrinus. Adult predators fed on few predator eggs and when they did no species preference was observed. 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, L. nigrinus and L. rubidus 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 greater on high prey-density branches than on low prey-density branches. 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 coexist and can be released in the same location for biological control of A. tsugae.

KEY WORDS biological control, Laricobius, competition, predation

Hemlock woolly adelgid, Adelges tsugae Annand (Hemiptera: Adelgidae), is a major pest of eastern hemlock, Tsuga canadensis (L.) Carrière, and Carolina hemlock, Tsuga caroliniana Engl. and Engleman, in the eastern United States. Adelgid nymphs penetrate plant tissues with their styllets to feed on the parenchyma cells that serve as nutrient transfer and storage cells in the xylem rays (Young et al. 1995). High density infestations lead to reduction of new shoot growth, shoot death, and eventual death or decline of the tree (McClure 1991, Young et al. 1995, Mayer et al. 2002). A multipronged research effort is underway to develop effective management strategies, such as breeding resistance in individual trees (Cowles et al. 2006), and biological control for large-scale, long-term sustainable management (Cheah et al. 2004). Ongoing efforts involve establishing a complex of predators. Such an effort requires knowledge of interspecific or intraguild competition among predators (Spiller 1986, Heinz and Nelson 1996, Losey and Denno 1998, Rosenheim 2001, Onzo et al. 2004). Flowers et al. (2005, 2006) measured the interaction among three heterospecific predator species of A. tsugae and found no negative competitive interactions among the species.

Three species of Laricobius have been discovered as potential biological control agents of A. tsugae: L. nigrinus Fender, L. rubidus LeConte, and L. osakensis Montgomery and Shiayki. All species in the genus Laricobius Rosenhauer (Coleoptera: Derodontidae) prey on Adelgidae, which feed only on Pinaceae (Lawrence and Hlavac 1979, Zilahi-Balogh 2001, Havill and Footitt 2007). Laricobius nigrinus is native to western North America, preying on A. tsugae assoicated with T. heterophylla (Raf. Sarg.) and T. mertensiana (Bong.) Carr. (Zilahi-Balogh et al. 2002, 2003; Lamb et al. 2005a; Mausel et al. 2010). Laricobius rubidus is native to eastern North America, where it is a predator of pine bark adelgid, Pineus strobi (Hartig), found on white pine, Pinus strobus L. (Clark and Brown 1960). Laricobius rubidus has taken advantage of the abundance of A. tsugae on hemlocks and is now commonly found feeding on A. tsugae, especially where white pines and hemlock occur together (Montgomery and Lyon 1996, Wallace and Hain 2000, Zilahi-Balogh et al. 2005, Mausel et al. 2008). Laricobius nigrinus is a specific predator of A. tsugae found

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in the western United States. It was first introduced for the control of *A. tsugae* in the eastern United States in 2003 (Mausel et al. 2010). *Laricobius osakensis*, discovered in *T. seiboldii* in 2005 (Montgomery et al. 2011), was imported into the United States in 2006 and studied under quarantine (Vieira et al. 2011). It was approved for release from quarantine by USDA, APHIS in 2010, and is currently being reared in anticipation of its initial field release.

An understanding of the competitive interactions among the three *Laricobius* species is essential in measuring their compatibility as part of a biological control program. The information gathered 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 or in sleeve cages placed in the field.

**Materials and Methods**

**Collection, Maintenance, and Sexual Determination of Beetles.** Adult beetles used in laboratory experiments were collected from the field in the fall of 2007 and 2008. *Laricobius rubidus* was collected from white pine, *P. strobulus* L. stands in Montgomery County, VA and Boone County, NC; *Laricobius nigrinus* was collected from western hemlock, *T. heterophylla*, in the greater Seattle, WA area; and *L. osakensis* was collected from the Kobe Arboretum near Osaka, Japan by using beat sheets. *Laricobius* larvae used in the laboratory experiments were offspring from collected beetles. Field experiments were carried out from fall 2008 to spring 2009 by using conspecific and congeneric groups of *L. nigrinus* and *L. rubidus* collected from the same sites as the specimens used in the laboratory studies.

Predator species were maintained in the laboratory under normal developmental conditions by using methods described by Lamb et al. (2005b). They were kept in 2.2-liter plastic containers ventilated with fine polyester mesh (Sefar, Depew, NY). Each container held no >20 adults and 5–7 *A. tsugae* infested *T. canadensis* clippings to facilitate larval development. Before laboratory experiments, oviposition tests were conducted to determine the sex of each adult beetle. One *Laricobius* adult was placed in individual nine by 2.5-cm polystyrene petri dishes containing *A. tsugae* infested *T. canadensis* branch clippings. After 72 h, the presence or absence of eggs in the oviposition arena was determined. Ovipositing females then were used in adult laboratory assays. Branch clippings were removed from the 2.2-liter plastic rearing containers as needed and searched to obtain adult or larval predator life stages for experimental assays. 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 three species (Zilahi-Balogh et al. 2005, Lamb et al. 2008). Containers were maintained in an environmental chamber (Percival-Scientific, Perry, IA) at 12°C and a photoperiod of 12:12 (L:D) h. All adult *Laricobius* were collected in the field at approximately the same time, and therefore, were of the same age.

**Laboratory Experiments**

**Individual Adult Assays.** This study was designed to get a baseline level for egg production, predation, and predator survival of each species. It was done by placing one adult female *Laricobius* in 9- by 2.5-cm polystyrene petri dish with clippings of *T. canadensis* infested with 60 *A. tsugae* ovisacs. The petri dishes were placed into an environmental chamber set at 12°C and a photoperiod of 12:12 (L:D) h 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. *Laricobius rubidus* enters peak egg production slightly later in the year than either *L. osakensis* or *L. nigrinus* (Clark and Brown 1960, Zilahi-Balogh et al. 2005, Lamb et al. 2008). 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.

**Conspecific and Congeneric Groupings of Adult or Immature Predators.** Egg production, predation, and predator survival were determined while adult predators were in conspecific and congeneric groupings. In the adult predators assays, three females of the same species or one female of each species (three total) were placed in a petri dish containing 15–20 cm of *T. canadensis* infested with 80 *A. tsugae* ovisacs. In the immature predators assays, each petri dish contained 80 *A. tsugae* ovisacs and three third-larval instars of the same species or one third instar of each species (three total). All petri dishes were placed into an environmental chamber set at 12°C and a photoperiod of 12:12 (L:D) h for 6 d. There were 10 replicates for each species or grouping. Each adult female or third instar was marked with a small amount of nontoxic water based opaque paint (Marvy Opaque Stix, Uchida of America Corp., Torrance, CA) to indicate species. 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 equals alive, 0 equals dead for all species within each group.

**Predation of Predator Eggs by Adult Laricobius.** Predation of predator eggs and *A. tsugae* ovisacs by adult *Laricobius* species at two prey-densities was determined by using choice tests. Choice tests contained 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, because of a lack of available *L. rubidus* eggs. Experiments containing *L. rubidus* were analyzed separately. Trials 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, and two eggs from each species (four eggs total). The 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. Each *T. canadensis* clipping was marked with a small piece of differently colored tape indicating egg species for all experiments. One adult *Laricobius* female was placed in each petri dish. Trials 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, and two eggs from each species (six eggs total). 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. The number of conspecific or congeneric predator eggs consumed by each species, predator eggs produced, sistentes ovisacs preyed upon, and survival were counted after 5 d. Predator survival was recorded as a binomial response: 1 = alive and 0 = dead.

A general linear model with type III fixed effects was used to analyze data. Tukey’s HSD test was used to separate means adjusted for multiple comparisons by using Bonferroni /H11021 Field Studies. Field experiments were conducted during the fall of 2009 by using sleeve cages described by Flowers et al. (2006) to measure predation, egg production, and survivorship of adult *L. nigrinus* and *L. rubidus* in both conspecific and congeneric groupings on preovipositing and ovipositing stages of *A. tsugae* (sistentes) at low (<90 ovisacs) and high (>120 ovisacs) densities. *Laricobius osakensis* was not included because it was still in quarantine at the time. Natural eastern hemlock stands with moderate to high *A. tsugae* infestations were selected in southwest Virginia and southeast Kentucky as case study sites. *Adelges tsugae* overwinters as developing nymphs (preoviposition stage) where ovisacs with no eggs are present. In early spring *A. tsugae* adults enter the oviposition stage. Although *Laricobius* adults survive on *A. tsugae* nymphs and adults, their larval progeny require *A. tsugae* eggs (Lamb et al. 2005b). *Laricobius nigrinus* and *L. osakensis* females are phenologically synchronized with their prey and do not begin depositing eggs in the ovisacs until *A. tsugae* begins oviposition (Zilahi-Balogh et al. 2003, Mausel et al. 2008). Preoviposition experiments were conducted twice at Mountain Lake (Giles County, VA) (37° 21’ 48.29” N, 80° 33’ 03.93” W, elevation 1,237 m), once in January (2009) and once in February (2009). Oviposition experiments were conducted twice in Kentucky Ridge State Forest (Bell County, KY) (36° 45’ 59.66” N, 83° 47’ 02.43” W, elevation 405 m), in April 2009. Kentucky Ridge State Forest was selected to continue experiments because of the rapid decline of hemlock health in the Mountain Lake area. The gender of *Laricobius* adults used in these experiments was not determined.

A generalized randomized complete block design blocked by date was used with four replications. The complete experiment was conducted twice at Mountain Lake and twice at Kentucky Ridge State Park. On each date, five *T. canadensis* trees with moderate to high *A. tsugae* densities were selected randomly. 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 by 0.6 m, and *A. tsugae* 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 nonpredator treatment control. These were superimposed on the two *A. tsugae* density levels. Baseline counts of *A. tsugae* 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. A fine-mesh polyester fabric cage (0.5 by 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, with either a conspecific or congeneric predator treatment or nonpredator control. Conspecific groups contained either two *L. nigrinus* or two *L. rubidus* per cage, whereas 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 by using cinch ties. Caged control branches were measured at both low and high ovisac densities to account for mechanical mortality of *A. tsugae* 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. Survivorship of adult predators was recorded as a binomial response (alive = 1; dead = 0). Branch clippings were examined
Table 1. Mean no. ± SE of predator eggs produced, ovisacs preyed upon, and percent survival of individual adult female Laricobius after 6 d in laboratory assays

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Mean eggs produced ± SE</th>
<th>Mean ovisacs preyed upon ± SE</th>
<th>% survivorship</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. nigrinus</td>
<td>7</td>
<td>6.8 ± 2.5a</td>
<td>19.2 ± 3.5a</td>
<td>70a</td>
</tr>
<tr>
<td>L. osakensis</td>
<td>9</td>
<td>11.0 ± 3.6a</td>
<td>23.0 ± 1.7a</td>
<td>90a</td>
</tr>
<tr>
<td>L. rubidus</td>
<td>8</td>
<td>0.8 ± 0.3b</td>
<td>15.0 ± 3.0a</td>
<td>90a</td>
</tr>
</tbody>
</table>

a Means within columns followed by different letters are significantly different (Tukey’s HSD test; P < 0.05).

Field experiments used 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, hemlock wooly adelgid oviposition stage, and date.

The general linear model (PROC GLM; SAS Institute 2009) included predator species combination, A. tsugae 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).

Data were transformed to achieve normality and equality of variances by using the Newton–Raphson method and means were separated using Tukey’s HSD test at P < 0.05.

Results and Discussion

Laboratory Experiments

Individual Assays. Significant differences in egg production were found among the three Laricobius species (F = 7.75; df = 2.21; P = 0.01). Laricobius rubidus produced fewer eggs over 6 d than L. nigrinus and L. osakensis (Table 1). Although all adult Laricobius species oviposited in prey ovisacs, very few eggs were oviposited by L. rubidus. Laricobius rubidus generally is active from March to June (Zilahi-Balogh et al. 2005), L. nigrinus from October to June (Zilahi-Balogh et al. 2003), and L. osakensis from October to June (Lamb et al. 2008). 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 may be less than that of the other two species because it is phenologically synchronized with P. strobi, its primary host, instead of A. tsugae (Zilahi-Balogh et al. 2005). Predation on ovisacs was not significantly different among species (F = 0.96; df = 2.21; P = 0.40), ranging from three to nearly four 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). This study suggests that at 12°C all three species are active.

Congeneric and Conspecific Adult Assays. Predator egg production, ovisacs preyed upon, and percent survival of adult Laricobius in conspecific or congeneric groups after 6 d in laboratory assays

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Mean eggs produced ± SE</th>
<th>Mean ovisacs preyed upon ± SE</th>
<th>% survivorship</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. nigrinus</td>
<td>10</td>
<td>24.0 ± 1.6a</td>
<td>35.0 ± 2.5b</td>
<td>100a</td>
</tr>
<tr>
<td>L. osakensis</td>
<td>9</td>
<td>26.0 ± 4.0a</td>
<td>45.2 ± 2.6a</td>
<td>90a</td>
</tr>
<tr>
<td>L. rubidus</td>
<td>7</td>
<td>11.3 ± 2.5b</td>
<td>27.0 ± 8.0a</td>
<td>70a</td>
</tr>
</tbody>
</table>

Means within columns followed by different letters are significantly different (Tukey’s HSD test; P < 0.05).
Table 3. Mean number ± SE of ovisacs preayed upon and percent survivorship of Laricobius larvae in conspecific or congeneric groups after 6 d in laboratory assays

<table>
<thead>
<tr>
<th>Larvae species</th>
<th>Mean ovisacs preayed upon ± SE</th>
<th>% survivorship</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 L. nigrinus</td>
<td>37.0 ± 6.0 aba</td>
<td>71a</td>
</tr>
<tr>
<td>3 L. osakensis</td>
<td>61.0 ± 5.5a</td>
<td>77a</td>
</tr>
<tr>
<td>3 L. rubidus</td>
<td>26.0 ± 5.0b</td>
<td>50a</td>
</tr>
<tr>
<td>1 L. nigrinus and L. rubidus</td>
<td>32.3 ± 6.5b</td>
<td>66a</td>
</tr>
<tr>
<td>1 L. osakensis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Means within columns followed by different letters are significantly different (Tukey’s HSD test; P < 0.05).

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

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

<table>
<thead>
<tr>
<th>Adult Laricobius spp.</th>
<th>Egg species</th>
<th>Mean no. Laricobius eggs consumed ± SE</th>
<th>% survivorship</th>
</tr>
</thead>
<tbody>
<tr>
<td>High prey density</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. nigrinus</td>
<td>L. nigrinus</td>
<td>0.11 ± 0.11ab</td>
<td>50a</td>
</tr>
<tr>
<td>L. osakensis</td>
<td>L. nigrinus</td>
<td>0.11 ± 0.11a</td>
<td>50a</td>
</tr>
<tr>
<td>L. nigrinus</td>
<td>L. osakensis</td>
<td>0.22 ± 0.22a</td>
<td>50a</td>
</tr>
<tr>
<td>L. osakensis</td>
<td></td>
<td>0.44 ± 0.24a</td>
<td>50a</td>
</tr>
<tr>
<td>Low prey density</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. nigrinus</td>
<td>L. nigrinus</td>
<td>0.22 ± 0.22a</td>
<td>50a</td>
</tr>
<tr>
<td>L. osakensis</td>
<td>L. nigrinus</td>
<td>0.33 ± 0.23a</td>
<td>50a</td>
</tr>
<tr>
<td>L. nigrinus</td>
<td>L. osakensis</td>
<td>0.78 ± 0.28a</td>
<td>50a</td>
</tr>
<tr>
<td>L. osakensis</td>
<td></td>
<td>0.55 ± 0.24a</td>
<td>50a</td>
</tr>
<tr>
<td>L. rubidus</td>
<td>L. nigrinus</td>
<td>0.20 ± 0.20ab</td>
<td>50a</td>
</tr>
<tr>
<td>L. osakensis</td>
<td>L. rubidus</td>
<td>0.50 ± 0.40a</td>
<td>50a</td>
</tr>
<tr>
<td>L. rubidus</td>
<td></td>
<td>0.50 ± 0.40a</td>
<td>50a</td>
</tr>
</tbody>
</table>

* Means within columns (for each prey density) followed by different letters are significantly different (Tukey’s HSD test; P < 0.05).

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 an additive predation rate. Survivorship was high for all species, suggesting that experimental conditions were suitable and negative interactions were low (Table 2).

**Conspecific and Congeneric Larval Assays.** Experiments were conducted to determine ovisac predation and survivorship of Laricobius larvae when in conspecific and congeneric groups. 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 all species or by L. rubidus only was significantly less (about half), compared with predation by L. osakensis only. The low net predation of the combined species treatment can be attributed to the fact that L. osakensis died in the combined treatments because of 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).

**Predation of Predator Eggs by Adult Laricobius When in the Presence of Two Prey Densities.** Experiments examining predation on predator eggs and sistens ovisacs by adult Laricobius species at two prey-densities were conducted in the laboratory. The model did not identify a significant interaction between main effects (F = 0.83; df = 1,6; P = 0.36). Therefore, the 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).

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 4). Values for all species were <1.

Predation on predators’ eggs was reduced but consistent across densities. Flowers et al. (2005, 2006) found an inverse relationship 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 preayed 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 coexisting predator species will have a limited impact on predator egg production through predation of predator eggs (Tables 4). Predation on predator eggs may be greater in these laboratory experiments than in a field environment because of the limited amount of space available for daily activities and the increased probability of finding eggs.

**Predation on Frey Ovisacs when Offered Two Prey-Densities.** Counts of prey ovisacs consumed did not differ significantly among Laricobius spp. adults (F = 0.16; df = 2,3; P = 0.85); however, net predation by both L. nigrinus and L. osakensis increased by almost 50% 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, whereas 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).
Higher densities of prey led to increased ovisac predation by all species. This density-dependent functional response is consistent with field observations. 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 L. nigrinus egg production was 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 because of available space. Lack of a numerical response among predators may have been because of 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.

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. Temperature requirements are important to all aspects of insect development, feeding, and greatly influences egg production (Cheah and McClure 1998, Stathas et al. 2001, Zilahi-Balogh et al. 2002). 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 because of 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.

### Field Experiments

#### Predation on Prey Ovisacs.
Because A. tsugae density provided to predators at the high-density level on 2 February was inconsistent with other dates, these data points were removed from analysis. No significant differences were found among dates. As a result, data were pooled over the four dates. Significant differences in net predation on prey ovisacs were found among sleeve cage treatments at both low and high prey-densities (Table 5). All caged control branches had significantly less predation than branches containing conspecific groups or congeneric (mixed) 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 a predator treatment by prey-density interaction (F = 3.53; df = 3.6; P = 0.0173). This interaction occurred because predation in the control treatment did not increase with increased prey-density, whereas 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 = 28.01; 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, whereas 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 74 and 58 ovisacs over 6 d, respectively. Congeneric groups of Laricobius predators preyed upon 81 ovisacs over 6 d.

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 A. tsugae in the spring when the combined actions of predator larvae and adults occur. In the current experiments, higher pest densities were associated with increased predation by both Laricobius species. Predation for each species combination almost doubled with presence of greater prey density, representing a density-dependent functional response.
January was significantly lower from the other dates. April, predator eggs were produced by either conspecific and conspecific groups were not observed. Negative interactions among congenic and conspecific treatments did support previous work by Zilhi-Balogh et al. Overall, the experiments did increase through time. If cage egg production was fairly low throughout these experiments but did not differ significantly among the predator treatments. The significant effect of date on oviposition illustrates the transition of Laricobius from preoviposition to oviposition stage. During the first two dates, A. tsugae had not yet matured to lay eggs. By April, A. tsugae adults were ovipositing. Virtually no predator eggs were produced by either Laricobius species before A. tsugae eggs were present. Oviposition by the predators was observed only when prey eggs were present in April.

In contrast with laboratory experiments, egg production did not differ significantly among the predator treatments. The significant effect of date on oviposition illustrates the transition of Laricobius from preoviposition to oviposition stage. During the first two dates A. tsugae had not yet matured to lay eggs. By April, A. tsugae adults were ovipositing. Virtually no predator eggs were produced by either Laricobius species before A. tsugae eggs were present. 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 A. tsugae and its Laricobius predators.

Survivorship. Predator survivorship was fairly high throughout all field experiments and ranged from 57 to 75% (Table 5). 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 likely was caused by very low temperatures. Throughout the coldest temperatures a high proportion of Laricobius predators’ survived. Negative interactions among congenic and conspecific groups were not observed.

Mean temperatures were between 5 and 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 placed on predator dispersal to search for more favorable conditions, especially during cold weather.

The data reported here provide insight into the presence and absence of interspecific and intraspecific competition. Although 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 coexisting, and are compatible from the perspective of predation of prey and oviposition of progeny. Mating attempts have also been observed among these predator species, further supporting a nonantagonistic relationship. Havill et al. (2011), reported that L. nigrinus and L. rubidus hybrids are being found in nature beyond the F1 generation. It is unknown what the implication of this finding is to the biological control effort and it is currently being investigated.

Both laboratory and field studies give indication that the three Laricobius species appear compatible in terms of predation of target prey, oviposition in prey ovisacs, and limited predation on each other.

### References Cited


Clark, R. C., and N. R. Brown. 1960. Studies of predators of the balsam woolly aphid, Adelges piceae (Ratzburg) (Ho-

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**Table 6.** Mean number ± SE Laricobius eggs (pooled by species treatment) deposited by date after 1 wk in field sleeve cage studies during preoviposition and oviposition stages of their prey, A. tsugae

<table>
<thead>
<tr>
<th>Date</th>
<th>n</th>
<th>Mean no. predator eggs deposited ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 January 2009</td>
<td>30</td>
<td>0.0 ± 0.00a</td>
</tr>
<tr>
<td>2 February 2009</td>
<td>30</td>
<td>0.06 ± 0.00b</td>
</tr>
<tr>
<td>11 April 2009</td>
<td>29</td>
<td>3.24 ± 0.53a</td>
</tr>
<tr>
<td>19 April 2009</td>
<td>29</td>
<td>3.13 ± 0.33a</td>
</tr>
</tbody>
</table>

* Means within columns followed by different letters are significantly different (Tukey’s HSD test; P < 0.05).


Stathas, G. J., P. A. Eliopoulos, D. C. Kontodimas, and J. Giannopapas. 2001. Parameters of reproductive activity...


Received 28 November 2011; accepted 29 May 2012.