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Competitive interactions among two specialist predators and a generalist predator of hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae) in south-western Virginia

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Abstract

1 Competitive interactions among two specialist predators, *Laricobius nigrinus* and *Sasajiscymnus (Pseudoscymnus) tsugae*, and a generalist predator, *Harmonia axyridis* Pallas, of hemlock woolly adelgid, *Adelges tsugae* were evaluated in hemlock stands in south-western Virginia. The two specialist predators are part of a biological control program for *A. tsugae*, and the potential for competition among these species and previously established generalist predators in the field is unknown.

2 Adult predators were evaluated in branch cages during spring and summer at two field sites infested with *A. tsugae*. Using females only in 2003 and sexual pairs in 2004, predator survival and net reproduction were examined, as well as their feeding and impact on *A. tsugae* when present alone and in conspecific and heterospecific groupings.

3 Predator survival was not affected by the presence of additional predators. Total feeding was greater for all species when placed in predator groupings, suggesting that interactions do not significantly interfere with feeding activity. Net reproduction per predator was negatively affected by conspecifics, but unaffected by heterospecifics, indicating that direct or indirect intraspecific interference may occur. In spring, *L. nigrinus* showed the greatest impact on *A. tsugae*, and *H. axyridis* had the greatest impact during summer.

4 These results suggest that it would be beneficial to utilize multiple predator species combinations over single species when implementing biological control for *A. tsugae*. Low-density releases are also recommended to reduce intraspecific interference.

Keywords Biological control, competitive interactions**,** *Harmonia axyridis***,** hemlock woolly adelgid**,** *Laricobius nigrinus***,** *Sasajiscymnus (Pseudoscymnus) tsugae***.**

Introduction

The hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae), is a major pest of eastern hemlock, *Tsuga canadensis* L. Carriere, and Carolina hemlock, *T. caroliniana* Engelmann, in the eastern United States. Heavily infested trees exhibit poor crown health and reduced shoot growth that, in combination with other environmental stresses, can result in rapid tree decline and death (McClure, 1987; Mayer *et al.*, 2002; Orwig *et al.*, 2002). In ornamental and nursery settings, systemic insecticides have proven effective in managing *A. tsugae* populations (McClure, 1992a; Rhea, 1995; Fidgen *et al.*, 2002; Webb *et al.*, 2003); however, forest hemlocks are found in many inaccessible locations or along riparian zones where chemical controls are not feasible. Because only generalist pathogens have been identified with *A. tsugae* (Reid *et al.*, 2002) and no known parasitoids are associated with Adelgidae (Montgomery & Lyon, 1996), biological control efforts using prey-specific predators have been initiated. Recent surveys in the eastern United States indicated that native predators consist primarily of generalists (Wallace & Hain, 2000), and that these species do not effectively control *A. tsugae* populations (Montgomery & Lyon, 1996). Classical biological control using predators from western North America and Asia is therefore underway (Cheah *et al.*, 2004).

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In North America, *A. tsugae* produce two parthenogenetic generations annually on hemlock (McClure, 1987). The sistens are present from summer to the next spring. In June and July, sistens nymphs emerge as crawlers from eggs and settle at the base of young needles where they immediately enter aestivation (summer diapause) (McClure, 1987; Salom *et al.*, 2001). They resume development in October and reach maturity in late winter. Adult sistens produce eggs from March until May, with nymphs developing into winged and wingless forms. The winged (sexupara) form disperses; however, no suitable alternate hosts are known in eastern North America (McClure, 1992b). The wingless form, the progrediens, remains on hemlock, reaching maturity and depositing sistens eggs in June (McClure, 1989).

Laricobius nigrinus Fender (Coleoptera: Derodontidae), native to the western United States and Canada, is a prey-specific predator of *A. tsugae* (Zilahi-Balogh *et al.*, 2002). Adults are black, have body lengths of ≤ 3 mm, and feed on all stages of *A. tsugae* sistens (Zilahi-Balogh *et al.*, 2002). Females oviposit singly within sistens ovisacs from January to May. Larvae feed primarily on *A. tsugae* eggs, and have four instars, during which time body length is increased from 1.7 to 3.6 mm. Mature larvae drop to the soil to pupate (Zilahi-Balogh *et al.*, 2003a). This species has good phenological synchrony with *A. tsugae* (Zilahi-Balogh *et al.*, 2003b). Emergent adults remain in the soil in aestival diapause during summer, and resume activity in the fall when sistens begin to develop. Development from egg to adult requires 666.7 degree-days (DD) above a lower developmental threshold of 3.7 °C (Zilahi-Balogh *et al.*, 2003c). Field studies indicate that *L. nigrinus* significantly reduces *A. tsugae* abundance within temporary branch enclosures, and can survive and reproduce in south-western Virginia from November to April (Lamb *et al.*, 2005). *Laricobius nigrinus* is univoltine and is being successfully mass reared in the laboratory (Lamb *et al.*, 2002). Open releases in hemlock stands in the eastern United States began in 2003.

Sasajiscymnus (Pseudoscymnus) tsugae Sasaji & McClure (Coleoptera: Coccinellidae), native to Japan, also appears to be a prey-specific predator of *A. tsugae* (Cheah & McClure, 1998; Butin *et al.*, 2002). Adults are black, have body lengths of 1.5–2.3 mm, and feed on all stages of sistens and progrediens (Sasaji & McClure, 1997). Females oviposit singly in concealed locations within curled bud-scales, in empty male cones, or beneath *A. tsugae* ovisacs (Cheah & McClure, 1998). Larvae feed on *A. tsugae* eggs, and have four instars, during which time body length is increased from 1.1 to 2.7 mm. Mature larvae pupate on the host tree. Development time from egg to adult requires 405 DD above a lower developmental threshold of 9.5 °C (Cheah & McClure, 2000). This species has a reproductive diapause that coincides with sistens aestivation (Cheah & McClure, 2000), and is capable of producing successive generations in the laboratory (McClure & Cheah, 1999; Palmer & Sheppard, 2002). To date, more than one million beetles have been released in hemlock stands in the eastern United States, and field collections in Pennsylvania and Connecticut suggest that this predator is established within these regions (Blumenthal, 2002; Cheah & McClure, 2002).

In addition to these newly introduced specialist species, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) was previously introduced for biological control of various hemipteran pests and has quickly spread to many regions of North America (Koch, 2003). Adults are extremely variable in colouration, have body lengths of 4.9–8.2 mm, and are highly polyphagous, voracious predators (Gordon, 1985). Females oviposit on host tree substrates in groups of approximately 20–30 eggs (Takahashi, 1987). Larvae have four instars, during which time body length is increased from 1.9 to 10.7 mm, and are covered with many scoli (Sasaji, 1977; Rhoades, 1996). Pupation occurs on the host tree. Development from egg to adult requires 267.3 DD above a lower developmental threshold of 11.2 °C (Lamana & Miller, 1998). This species is bivoltine in North America. On arrival of warmer temperatures in spring, adults mate and disperse from overwintering sites to find food and oviposition sites (Lamana & Miller, 1996; Koch & Hutchinson, 2003). In the south-eastern United States, surveys for natural enemies of *A. tsugae* found that predators were rare overall, and *H. axyridis* was the most abundant predator based on beat samples (Wallace & Hain, 2000).

Although this species is an important arboreal biological control agent of aphids in pecan (Larock & Ellington, 1996), apple (Brown & Miller, 1998) and citrus (Michaud, 2002), *H. axyridis* expansion into an area can dramatically affect local populations of aphidophagous predators. Declines in populations of the native, arboreal coccinellids *Brachiacantha ursine* F., *Cycloneda munda* Say and *Chilocorus stigma* Say in south-western Michigan (Colunga-Garcia & Gage, 1998) and *Cycloneda sanguinea* L. in Florida (Michaud, 2002) have been associated with invasion of *H. axyridis*. In addition to impacts on native species, *H. axyridis* has also replaced another established exotic species, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), as the predominant predator in arboreal habitats in western Oregon (LaMana & Miller, 1996) and West Virginia (Brown & Miller, 1998). The mechanisms of interference are not well understood, but 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).

The present study examined competitive interactions among two specialist predators, *L. nigrinus* and *S. tsugae*, which are being released as part of a biological control programme for *A. tsugae*, and a previously established generalist predator, *H. axyridis*, now common to infested hemlock stands. Previous short-term bioassays in the laboratory indicated that competitive interactions occur among conspecifics of these species, whereas heterospecifics showed non-interference (Flowers *et al.*, 2005). Therefore, the present study evaluated competitive interference in a field setting over longer durations and under more natural conditions.

Materials and methods

Insect cultures

Laricobius nigrinus adults were obtained from a colony at Virginia Polytechnic Institute and State University

(Blacksburg, Virginia). *Sasajiscymnus (Pseudoscymnus) tsugae* adults were obtained from a colony at the Philip Alampi Beneficial Insect Laboratory at the New Jersey Department of Agriculture (Trenton, New Jersey). *Harmonia axyridis* adults were obtained from Rincon-Vitova Insectaries (Ventura, California). All predators were reared under their normal developmental conditions at 10 °C, under an LD 12 : 12 h photoperiod at 75% relative humidity (RH) for *L. nigrinus*, and at 25 °C, under an LD 16 : 8 h photoperiod and 45% RH for both coccinellid predators. The coccinellid species were stored at 20 °C, under an LD 16 : 8 h photoperiod at 45% RH before shipment to our laboratory. Upon receiving the predators, conditions were gradually stepped down from 20 to 10 °C and from an LD 16 : 8 h photoperiod to LD 12 : 12 h over a 2–3-week period to precondition predators to existing field conditions. For preconditioning, all species were placed in 2.2-L plastic containers lined with moistened filter paper and ventilated with fine polyester mesh (PeCap®, Sefar, Kansas City, MO). Each container held 15 adults, in a female : male sex ratio of 2 : 1, and with five to seven *T. canadensis* branch clippings heavily infested with *A. tsugae*. Containers were maintained in an environmental chamber (model I-36, Percival-Scientific, Boone, IA). Adult predators were removed from holding containers 24 h before deployment to the field, and placed individually, along with branch clippings containing ten *A tsugae*, into 1 × 5 cm Petri dishes (Fisherbrand®, Fisher-Scientific, Hampton, NH) lined with moistened filter paper. All predators were mature, of approximately the same age (12–24 weeks), and were selected randomly from rearing containers. Predators were identified to sex using morphological characters of *H. axyridis* (Gordon, 1985) and *S. tsugae* (Sasaji & McClure, 1997) and by monitoring oviposition by *L. nigrinus*. During preconditioning, egg production by all species was evident in holding containers. Microscopic examination of a subset of the *A. tsugae*-infested foliage, which predators were placed on before deployment in the field, showed that predator eggs of each species were present. This confirmed that some females were reproductively active, and suggested that reproductive capabilities were not impaired by preconditioning, even though it was not possible to insure this for each female.

Experimental design of field studies

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

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

Predators remained in the enclosures for 6 weeks during the spring (1 April to 14 May) and 4 weeks during the summer (1 June to 1 July). This experimental duration represented the period when successful predator reproduction on *A. tsugae* could occur. This is due to the availability of *A. tsugae* eggs, which are essential for survival and growth of predator progeny. In late summer, first-instar sistens are in aestivation, an inappropriate stage for predator progeny development. Nine replications of each treatment and control were completed in 2003, and ten replications were completed in 2004. Two data loggers (Hobo®, Onset Computer, Pocasset, MA) were used to record stand conditions. Mean temperature and RH in 2003 were 10 ± 2 °C and 60% RH during the spring and 19 ± 3 °C and 75% RH during the summer. In 2004, stand conditions were 12 ± 2 °C and 60% RH and 24 ± 3 °C and 80% RH during the two evaluation periods, respectively. At the conclusion of each study, branches were cut and returned intact to the laboratory. The number of surviving adult and immature predator life stages, on each branch, were recorded. Branches were then sectioned, placed into 3.8-L freezer bags and stored at –20 °C. Branch clippings were examined by microscope 4–6 weeks later to obtain additional counts of predator net reproduction, and to assess predator feeding and impact on *A. tsugae*.

Evaluation of predator responses

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

Predator net reproduction, as well as feeding and impact on *A. tsugae*, were assessed by mircoscopic inspection of randomly selected clippings from each sectioned branch (total length: 200cm). Net reproduction was determined by counts of intact predator progeny, of all life stages. For conspecific treatments, the net reproduction per female predator was calculated. Eggs, larvae and pupae were distinguished using morphological characteristics of *L. nigrinus* (Zilahi-Balogh *et al.*, 2003a), *S. tsugae* (Sasaji & McClure, 1997) and *H. axyridis* (Gordon, 1985). Predator feeding was determined by counts of the number of sistens or progrediens ovisacs with evidence of predation (i.e. when the adelgid ovisac was disrupted and the adult or progeny within showed evidence of being fed upon or killed by a predator). Predator feeding was distinguished from other causes of mortality by the presence of puncture wounds or damage to the exoskeleton that was consistent with previously observed predator attacks. Predation on *A. tsugae* in each combination was assessed as a total for all predators in the treatment, as feeding by individual species could not be distinguished. To assess predator impact during the spring, counts of the number of intact, developing progrediens, remaining on branches at the completion of the study, were performed. Progrediens nymphs were judged to be alive and developing based on the presence of new woolly filaments. Counts of sexuparae were not included because they naturally disperse from branches (McClure, 1989, 1991). Predator impact during the summer was determined by counts of the number of aestivating sistens present on the new growth of the branch sections.

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

Predator net reproduction, feeding and impact on *A. tsugae* were examined separately by season, and analyses of net reproduction were further separated by species. For each analysis, a general linear model (PROC GLM; SAS Institute, 2001) was used that included predator species combination, year, and their interaction as independent categorical variables. The results for these responses were $\log_{10}$-transformed to achieve normality and equality of variances, and analysed using two-way analysis of variance (Zar, 1984). All analysis of variance was followed by Tukey's honestly significant difference test to separate treatment means (Zar, 1984).

Results

Predator survival

Survival of predators ranged between 61.1 and 83.3%. There was no interaction between the model variables, and each factor was evaluated separately. For each species, in both the spring and summer evaluations, there were no significant differences in survival that were related to predator species combination (Table 1). There were also no significant differences by year for *L. nigrinus* (Wald $\chi^2 = 0.51$, d.f. = 1, $P = 0.94$), *S. tsugae* (Wald $\chi^2 = 0.09$, d.f. = 1, $P = 0.77$), and *H. axyridis* (Wald $\chi^2 = 1.56$, d.f. = 1, $P = 0.21$) in the spring, or *S. tsugae* (Wald $\chi^2 = 0.13$, d.f. = 1, $P = 0.72$) and *H. axyridis* (Wald $\chi^2 = 0.28$, d.f. = 1, $P = 0.87$) in the summer.

Predator net reproduction

Analyses of predator net reproduction revealed no interaction between the variables in either season, so factors were considered independently. During the spring, there were significant differences by predator species combination for *L. nigrinus* ($F = 11.76$, d.f. = 4,85, $P < 0.0001$) (Fig. 1A) and *H. axyridis* ($F = 15.32$, d.f. = 4,85, $P < 0.0001$) (Fig. 1C), but not *S. tsugae* ($F = 0.87$, d.f. = 4,85, $P = 0.43$) (Fig. 1B). The highest net reproduction during this period was by *L. nigrinus*, whereas *S. tsugae* and *H. axyridis* each had relatively low levels of net reproduction in the spring by comparison. Progeny of *L. nigrinus* were also more advanced developmentally, predominantly occurring as larvae and prepupae, whereas the coccinellid species consisted of mostly egg and early larval life stages. All species had lower net reproduction per predator in conspecific groupings; however, it was only reduced to a statistically significant degree for *L. nigrinus* and *H. axyridis* (Fig. 1A, C). Heterospecific groupings

Table 1 Percent survival of female predators in branch cages with *A. tsugae* in spring and summer

Season	Predator species	Predator species combination	*n*	Survival (%)	Wald χ^2	*P* (prey stage)[a]
Spring (sistens)	*Ln*	Alone	17	76.5	0.05	0.97
		Ln + Ln	18	72.2		
		Ln + St	18	77.8		
		Ln + Ha	19	73.7		
		Ln + St + Ha	17	70.6		
	St	Alone	18	72.2	0.76	0.94
		St + St	19	73.7		
		St + Ln	19	78.9		
		St + Ha	17	70.6		
		St + Ln + Ha	19	68.4		
	Ha	Alone	19	68.4	0.21	0.96
		Ha + Ha	18	61.1		
		Ha + Ln	17	70.6		
		Ha + St	19	63.2		
		Ha + Ln + St	18	66.7		
Summer (progrediens)	*St*	Alone	18	77.8	0.15	0.92
		St + St	17	76.5		
		St + Ha	19	78.9		
	Ha	Alone	19	78.9	0.28	0.87
		Ha + Ha	19	73.6		
		Ha + St	18	83.3		

Predators were evaluated alone and in conspecific and heterospecific groupings. Values represent the combined results for 2003 and 2004. *Ln*, *Laricobius nigrinus*; *St*, *Sasajiscymnus tsugae*; Ha, *Harmonia axyridis*.

[a]No significant differences were found by predator combination for any species during either season.

did not significantly affect net reproduction by these species. During the summer evaluation, net reproduction was similar, by predator species combination, for *S. tsugae* ($F = 1.54$, d.f. = 2,51, $P = 0.21$) (Fig. 2A), and significantly different for *H. axyridis* ($F = 38.63$, d.f. = 2,51, $P < 0.0001$) (Fig. 2B). Consistent with the spring evaluation, *H. axyridis* had significantly reduced net reproduction in conspecific groupings, whereas heterospecific groupings showed no significant effect on either species. Net reproduction by year was similar for all predators in the spring: *L. nigrinus* ($F = 3.16$, d.f. = 1,85, $P = 0.08$), *S. tsugae* ($F = 3.46$; d.f. = 1,85; $P = 0.07$) and *H. axyridis* ($F = 3.02$, d.f. = 1,85, $P = 0.09$). The summer evaluation also showed no differences for *S. tsugae* ($F = 1.89$, d.f. = 1,51, $P = 0.17$) or *H. axyridis* ($F = 3.60$, d.f. = 1,51, $P = 0.07$).

Predator feeding on *A. tsugae*

Feeding by predators was also influenced by model variables, which again showed no significant interaction. Feeding was significantly different by predator species combination during the spring ($F = 430.40$, d.f. = 10,187, $P < 0.0001$) (Fig. 3A) and summer ($F = 172.41$, d.f. = 5,102, $P < 0.0001$) (Fig. 3B). In the spring, individual species comparisons showed that *L. nigrinus* and *H. axyridis* fed on significantly more sistens than *S. tsugae* and, in summer, *H. axyridis* fed on significantly more progrediens than *S. tsugae*. Comparisons of individual predator species, with their respective conspecific and heterospecific groupings revealed that total feeding was significantly higher for all species when placed in predator groupings. During the spring, the greatest amount of feeding occurred in the three-species treatment, whereas the conspecific grouping of *H. axyridis* had the highest amount of feeding during summer. All predator treatments showed significantly more feeding than control branches during each season. Analyses by year indicated that there was significantly more feeding in 2004 during the spring ($F = 38.35$, d.f. = 1,187, $P < 0.0001$) and summer ($F = 90.05$, d.f. = 1,102, $P < 0.0001$).

Predator Impact on *A. tsugae*

Analyses of predator impact on *A. tsugae* showed no interaction between the variables in either season, and the factors were considered again independently. There were significant differences by predator species combination in the spring ($F = 186.29$, d.f. = 10,187, $P < 0.0001$) (Fig. 4A) and summer ($F = 30.17$, d.f. = 5,102, $P < 0.0001$) (Fig. 4B). Comparisons among the three species showed that *L. nigrinus* had significantly greater impact than *S. tsugae* or *H. axyridis* during the

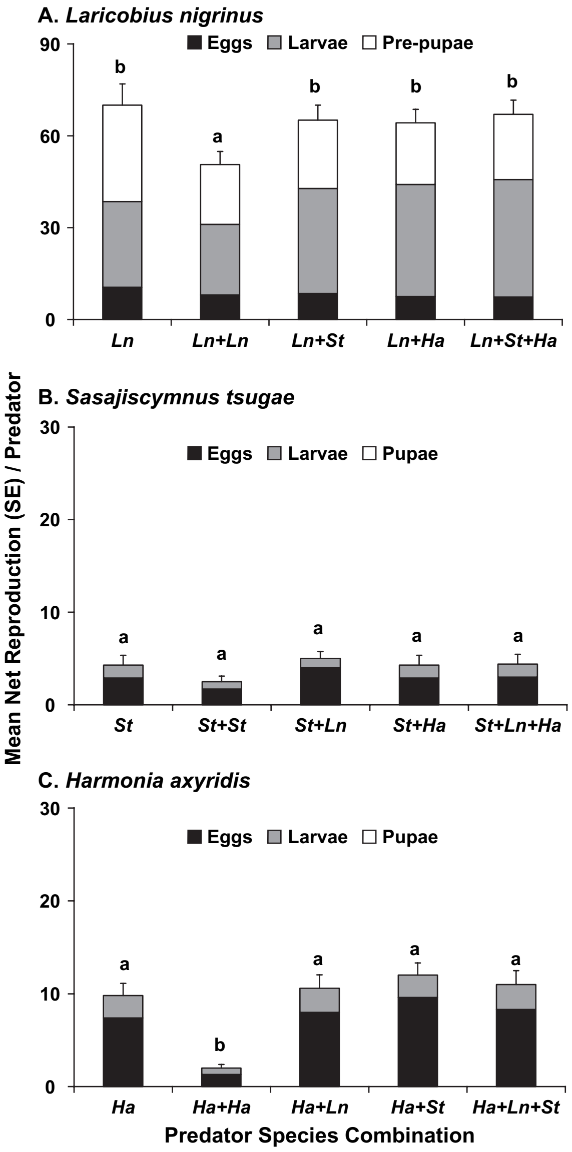

Figure 1 Mean ± SE net reproduction per female predator, by species combination, for *Laricobius nigrinus* (*Ln*) (A), *Sasajiscymnus tsugae* (*St*) (B) and *Harmonia axyridis* (*Ha*) (C) in the spring evaluation. Values represent the combined results for 2003 and 2004. The portion of predator progeny by life stage is indicated for each species. Means for columns with the same letter above are not significantly different.

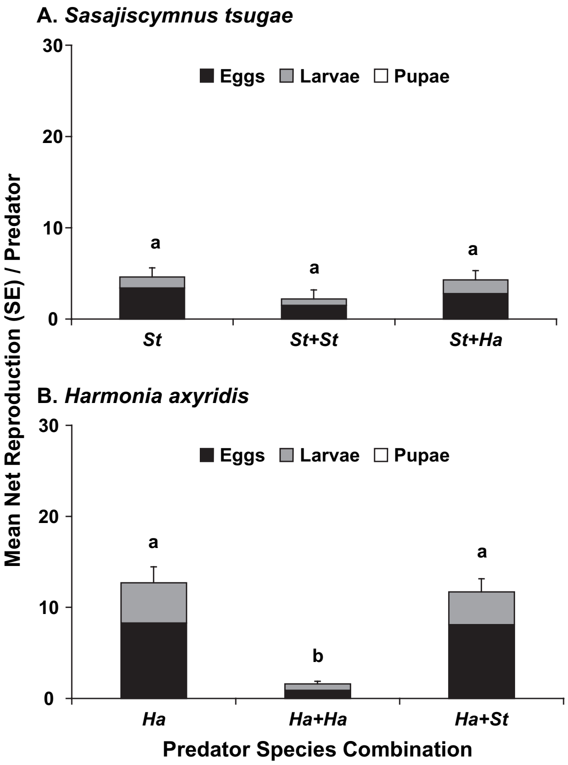

Figure 2 Mean ± SE net reproduction per female predator, by species combination, for *Sasajiscymnus tsugae* (A) and *Harmonia axyridis* (B) in the summer evaluation. Values represent the combined results for 2003 and 2004. The portion of predator progeny by life stage is indicated for each species. Means for columns with the same letter above are not significantly different.

spring, and *H. axyridis* had greater impact than *S. tsugae* during the summer. Comparisons among spring predator treatments revealed that *L. nigrinus* and *H. axyridis* had significantly greater impact with conspecifics, in heterospecific groupings with one another and with all three species. For *S. tsugae*, there was significantly greater impact in all heterospecific groupings, whereas conspecifics did not improve their impact. In the summer evaluation, results for *S. tsugae* again showed significantly improved impact in heterospecific groupings, whereas conspecifics did not improve results. For *H. axyridis*, impact was significantly improved by conspecifics, but not by the heterospecific combination. All predator treatments showed significantly greater impact than control branches during each season. Predator impact by year was similar in spring ($F = 1.17$, d.f. = 1,187, $P = 0.32$) and summer ($F = 1.23$, d.f. = 1,102, $P = 0.29$).

Discussion

Cannibalism and interspecific predation by adult females was not observed to a significant degree in these studies. Although *H. axyridis* has the potential to be a voracious intraguild competitor (Koch, 2003), the present study showed that predator survival was not impacted by direct competition. This was probably due to the high host densities of the branch enclosures. In addition, despite the low temperatures experienced during these trials, each species had high survival, providing further evidence that these predators are well adapted to surviving in *A. tsugae* infested hemlock stands in the south-eastern United States.

Although *L. nigrinus* had the highest net reproduction in the spring, previous studies have indicated that the coccinellid species have much higher lifetime mean fecundity in the laboratory (Cheah & McClure, 1998; Stathas *et al.*, 2001; Zilahi-Balogh *et al.*, 2003a). However, temperature requirements

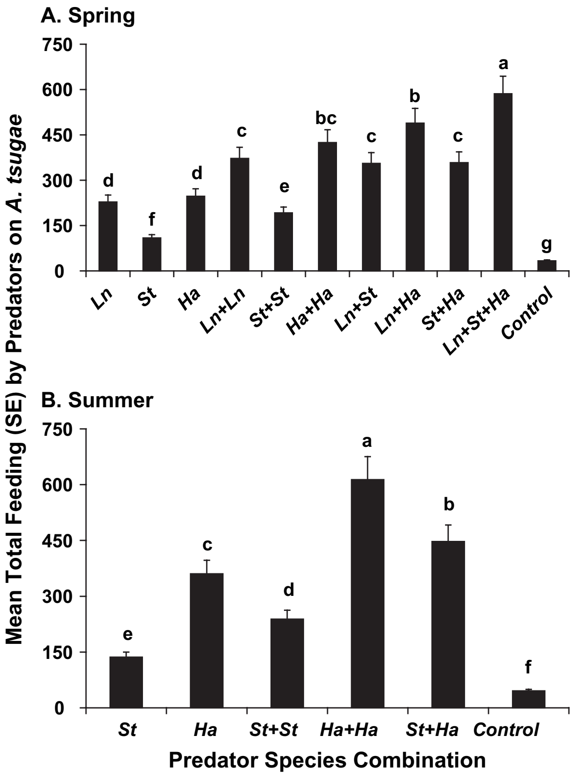

Figure 3 Mean ± SE total feeding by predators on *Adelges tsugae* sistens in spring (A) and progrediens in summer (B). Predator feeding was measured as the number of adelgid ovisacs that showed evidence of predation on adults or progeny. Values represent the combined results for 2003 and 2004. Means for columns with the same letter above are not significantly different.

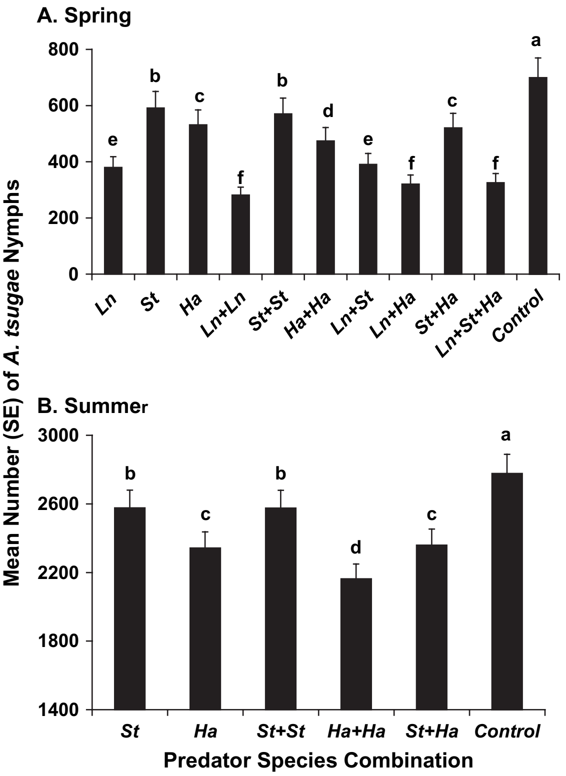

Figure 4 Mean ± SE number of developing *Adelges tsugae* progrediens (A) at the conclusion of the spring trial, and aestivating sistens (B) at the conclusion of the summer trial. Predator impact is indicated by adelgid density after predator feeding and reproductive activities. Values represent the combined results for 2003 and 2004. Means for columns with the same letter above are not significantly different.

are also much greater for the coccinellid species. The developmental threshold for *L. nigrinus* is lower than that of *S. tsugae* or *H. axyridis* by 5.8 and 7.5 °C, respectively (Cheah & McClure, 1998; LaMana & Miller, 1998; Zilahi-Balogh *et al.*, 2003c). Because reproduction by all species is influenced by temperature (Cheah & McClure, 1998; Stathas *et al.*, 2001; Zilahi-Balogh *et al.*, 2002), the lower threshold of *L. nigrinus* may have allowed increased reproduction at low temperatures. The progeny of *L. nigrinus* were also more advanced developmentally than the coccinellid species, indicating earlier reproductive activity. Temperatures and photoperiods during the summer evaluation were similar to optimal rearing conditions for the coccinellids; however, reproduction may still have been limited due to prey quality (Palmer & Sheppard, 2002), host tree health (Sheppard & Palmer, 2004) or nutrient limitation (Flowers *et al.*, 2005). Additionally, branch cages limited predator dispersal and choice of potentially more favourable microhabitats.

Reproductive interference by conspecifics was evident to varying degrees, consistent with previous laboratory studies (Flowers *et al.*, 2005). Negative effects were most apparent for *H. axyridis* and *L. nigrinus*, whereas *S. tsugae* displayed a lesser degree of interference. Previous studies of *H. axyridis* indicate that indirect reproductive interference occurs by passive substrate marking using faecal cues (Agarwala *et al.*, 2003) and oviposition-deterring pheromones (Yasuda *et al.*, 2000). Direct interference by egg cannibalism also occurs (Osawa, 1992; Flowers *et al.*, 2005), and provides increased nutrition and growth when prey is of low quantity or quality (Wagner *et al.*, 1999; Yasuda & Ohnuma, 1999; Snyder *et al.*, 2000; Michaud & Grant, 2004). In the present study, both direct and indirect competition occurred. Microscopic examination of branch clippings showed remnant chorions of *H. axyridis* eggs due to cannibalism. However, it appeared that oviposition did not occur initially in most cases, and that reproductive interference for *H. axyridis* was by indirect means. The chemical ecology exhibited by this species has not been investigated for the specialist predators, but similar mechanisms may be used. By contrast, heterospecific reproductive interference did not occur to a significant degree. Although predation of heterospecific progeny by *H. axyridis* is well documented (Yasuda & Shinya, 1997; Burgio *et al.*, 2002; Flowers *et al.*, 2005), it was limited in these studies because of high host densities and low temperatures. By contrast, chemical deterrents in eggs (Pasteels *et al.*, 1973) and defensive scoli of larvae (Dixon, 2000) may have prevented interspecific predation of *H. axyridis* life stages. Net reproduction was similar in both years of the study for spring and summer, indicating that environmental conditions may be the limiting factor for the coccinellid species, and not insufficient mating of females.

Feeding by predators was also greatly influenced by ambient temperatures. In the spring, the larger and more voracious *H. axyridis*, and smaller *L. nigrinus*, had similar amounts of feeding because of decreased activity by *H. axyridis* and higher reproduction by *L. nigrinus* at the low environmental temperatures (Lamb *et al.*, 2005). By contrast, predator feeding in summer was positively associated with body size, with the larger *H. axyridis* feeding more than *S. tsugae*. Feeding by the coccinellid species was greater in summer than in spring, supporting studies that show these predators to be more active during summer (Lamana & Miller, 1998; Cheah & McClure, 2000). The incorporation of additional conspecific and heterospecific predators significantly increased the total amount of feeding, in agreement with previous laboratory trials (Flowers *et al.*, 2005). It was not possible to determine whether per predator feeding average increased (additive effects), but the extent of predator interference did not have a negative effect on feeding activity. Predator feeding was greater in 2004, and this is probably attributable to the inclusion of male predators, as environmental conditions were similar. Limited feeding on *A. tsugae* on uncaged control branches indicates that native species are not affecting *A. tsugae* densities in hemlock stands.

Predator impact on *A. tsugae* population densities varied substantially between seasons. In spring, *L. nigrinus* had the greatest impact as a result of adult feeding and high net reproduction. The number of progeny produced in each trial consumed a large number of eggs in sistens ovisacs, thus limiting the growth of the progrediens generation. During summer, *H. axyridis* had the greatest impact on the progrediens and the number of aestivating sistens remaining, although the reductions in this season were lower by comparison. The coccinellid predators concentrated feeding primarily on *A. tsugae* adults in each season that had already produced eggs. This factor coupled with reduced reproduction led to a lower degree of impact on the next generation. Due to the amount of feeding and/or reproduction by *L. nigrinus* and *H. axyridis* during these trials, predator combinations involving these species had greater impact than *S. tsugae*, which appeared to display limited activity in these trials, and may be more sensitive to experimental or environmental conditions. In each season, predator treatments reduced the number of developing or aestivating *A. tsugae* nymphs relative to controls; however, it is unknown whether these reductions are sufficient to reduce future adelgid densities below injurious levels. Predator impact was similar by year during both seasons, indicating that it is the production of progeny and not additional adult predators that have the greatest impact on future *A. tsugae* generations.

Competitive interactions among predators may directly or indirectly affect predator diversity and biological control (Polis *et al.*, 1989; Rosenheim *et al.*, 1993; Lucas *et al.*, 1998). Initial field studies of these three predators of *A. tsugae* indicate that conspecific interference may negatively affect these species, whereas heterospecific interference was limited. However, the experimental design has many constraints because branch enclosure studies do not reflect the potential for predator immigration and emigration in response to changing environmental conditions or prey abundance. In addition, the level of competition among predators may have been constrained by the reduced activity of the coccinellid species under low temperature field conditions.

However, it is proposed that the present study accurately represents the conditions occurring during the seasonal overlap of these species, and the results suggest that significant interspecific competition does not occur. Overall, the lack of strong seasonal synchrony among these species may limit competition in the field. The seasonal overlap of *L. nigrinus* with coccinellid predators and other native generalists may be reduced because of their early spring development; however, *S. tsugae* may be at greater risk due to higher temperature requirements and summer development that overlaps with other generalist predators. In conclusion, we recommend using multiple predator combinations over single species when implementing biological control for *A. tsugae* because interspecific competition does not appear to be significant. Low-density predator releases are also recommended to reduce the potential negative effects of intraspecific interference.

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