

Behavior and daily activity patterns of specialist and generalist predators of the hemlock woolly adelgid, *Adelges tsugae*

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Abstract The behavior and daily activity patterns of two specialist predators, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) and *Sasajiscymnus tsugae*, Sasaji and McClure (Coleoptera: Coccinellidae), and a generalist predator, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), of hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), were examined using digital video recording in the laboratory. The two specialists are part of a biological control program for *A. tsugae*, and it is not known if competitive interactions with previously established generalist predators will negatively impact their effectiveness. The behavior and daily activity patterns of adult females of each species were documented in singley and pairedpredator assays under simulated spring and summer conditions. Behavior varied qualitatively and quantitatively by species, and did not appear to be highly coordinated temporally or spatially. All species exhibited continuous activity patterns that were punctuated by longer periods of rest. Extensive and intensive searching behavior occurred in all species, with intensive searching being highly variable. Specialist predators appeared to be more selective of feeding and oviposition sites, and rested at more concealed locations than the generalist species. In spring conditions, *L. nigrinus* had greater activity and a more even behavior distribution than *S. tsugae* or *H. axyridis*, which were skewed towards resting. In summer, the latter two species showed increased activity at higher temperatures. Conspecifics significantly altered the time allocated to specific behaviors for *L. nigrinus* and *H. axyridis*, resulting in reduced predator effectiveness by reducing time and energy expenditure on activities that directly impact the adelgids. In contrast, *S. tsugae* conspecifics and all heterospecific combinations showed nonyinterference. The activity of each species varied with time of day; *L. nigrinus* was more active at night, while *S. tsugae* and *H. axyridis* were more active during the day. All predator groupings maintained a high degree of spatial separation relative to assay size. The use of multiplepredator species combinations that include the specialist predators, is recommended over singleyspecies for biological control of *A. tsugae*, as temporal and spatial patterns were not highly coordinated. Lowydensity releases may reduce the potential negative effects of intraspecific competition.

Keywords: **Correspondence:** *Laricobius*^a rflowers@odf.state.or.us, *nigrinus*, *Sasajiscymnus*^b salom@vt.edu, *tsugae*, *Harmonia*^c tkok@vt.edu, *axyridis*, predator^d mullinsd@vt.edu
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Introduction

ical control efforts using preyyspecific predators

The hemlock woolly adelgid, *Adelges tsugae* have been initiated (Cheah et al. 2004).

Annand (Hemiptera: Adelgidae), is a major pest of eastern hemlock, *Tsuga canadensis* L. Carriere, and Carolina hemlock, *T. caroliniana* Engelmann, in the eastern United States. This species is believed to originate from southern Japan, where it is an innocuous inhabitant of *Tsuga spp.* (Havill et al. 2006).

Populations in these areas are likely regulated by host resistance and natural enemies (McClure et al. 2001). *Adelges tsugae* was first observed in the eastern United States in 1951 in Virginia (Stoetzel 2002), and has since spread south into the southern Appalachians and New England, causing widespread mortality of hemlock species (Cheah et al. 2004). 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). The tremendous population growth of this species in the eastern

Laricobius nigrinus Fender (Coleoptera: Derodontidae), native to the western United States and Canada, is a prey-specific predator of *A. tsugae* (ZilahiyBalogh et al. 2002) found on western hemlock *T. heterophylla* (Raf.) Sargent, a species not typically injured by *A. tsugae* (Furniss and Carolin 1977). Adults feed on all stages of *A. tsugae* (ZilahiyBalogh et al. 2002), and show good phenological synchrony with *A. tsugae* (ZilahiyBalogh et al. 2003a). Field studies indicate that *L. nigrinus* significantly reduces *A. tsugae* densities within temporary branch enclosures, and can survive and reproduce in southwest Virginia from November to April (Lamb et al. 2005a; 2006). This species has been mass reared in the laboratory (Lamb et al. 2005b), and released into hemlock stands in the eastern United States since 2003 (Mausel 2004; Lamb et al. 2006).

United States has been attributed to the absence of effective natural enemies (Cheah and McClure 1998; Wallace and Hain 2000), their ability to survive at low temperatures (Parker et al. 1999), and the high susceptibility of eastern hemlock species.

Sasajiscymnus (Pseudoscymnus) tsugae Sasaji and McClure (Coleoptera: Coccinellidae), native to Japan, is believed to be a specialist predator of *A. tsugae* (Cheah and McClure 1998, Butin et al. 2002). Adults feed on all stages of *A. tsugae* (Sasaji and McClure 1997), and females have a

In North America, *A. tsugae* is anholocyclic, reproducing only asexually on its secondary host, *Tsuga spp.* (McClure 1989). The overwintering generation, or sistens, is present from summer to the following spring (McClure 1989; 1991). Crawlers

emerge in April and May, with nymphs developing into wingless and winged forms. The wingless form, or progrediens, remains on reproductive diapause that coincides with *A. tsugae* aestivation (Cheah and McClure 2000). It is capable of producing successive generations in the laboratory (McClure and Cheah 1999; Palmer and Sheppard 2002). To date, over 1 million beetles have been released into hemlock stands in the eastern United States, and this predator is established within this region (Cheah et al. 2004).

hemlock and deposit eggs from which sistens crawlers emerge in June and July, settle at the base of young needles, and immediately enter summer diapause (aestivation) (McClure 1987; Salom et al. 2001). Sistens resume development in October and reach maturity in late winter; thus, two asexual generations are completed annually on hemlock. In ornamental and nursery settings, systemic insecticides have proven effective in managing *A. tsugae* populations (McClure 1992; Rhea 1995; Fidgeon et al. 2002; Webb et al. 2003); however, forest hemlocks are found in many inaccessible locations or along riparian zones where chemical controls are neither reasonable nor allowed. Since only generalist pathogens have been identified with *A. tsugae* (Reid et al. 2002) and no known parasitoids are associated with Adelgidae (Montgomery and Lyon 1996), biology

In addition to these newly introduced species, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), previously introduced from western Asia for biological control of various hemipteran pests, has quickly spread into many regions of North America (rev. in Koch 2003). Adults are polyphagous, voracious predators (Gordon 1985; Takahashi 1987; Majerus 1994). This species migrates to overwintering sites in late fall (Lamana and Miller 1996; Koch and Hutchinson 2003), and with warmer temperatures in spring, they mate and disperse from these sites (Lamana and Miller 1996). In the southeastern United States, surveys for natural enemies of *A. tsugae* showed that, while predators were generally scarce overall, *H. axyridis* was the most abundant predator collected from branch beat samples (Wallace and Hain 2000).

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Although *H. axyridis* is an important arboreal biological control agent of aphids in pecan (Larock and Ellington 1996), apple (Brown and Miller 1998) and citrus (Michaud 2002), its expansion into an area can dramatically affect local populations of aphidophagous predators. Declines in the populations of native, arboreal coccinellids, *Brachiacantha ursine* F., *Cycloneda munda* Say and *Chilocorus stigma* Say in southwestern Michigan (ColungayGarcia and Gage 1998) and *Cycloneda sanguinea* L. in

patterns of these species, focusing on extensive and intensive searching, feeding, resting, and oviposition behaviors.

Two sets of experimental conditions were used to provide greater coverage over the lifecycle of *A. tsugae* and better approximate the natural conditions under which these interactions may occur. In addition, the effects of intray and interspecific competition on predator temporal and spatial patterns were examined to more thoroughly examine predator compatibility in this system. Florida (Michaud 2002), have been associated with invasion by *H. axyridis*.

In addition to

Materials and Methods impacts on native species, *H. axyridis* has also

Insect cultures replaced another established exotic species,

L. nigrinus adults were obtained from a colony *Coccinella septempunctata* L. (Coleoptera: reared at Virginia Polytechnic Institute and State Coccinellidae), as the predominant predator in University (Blacksburg, Virginia). *S. tsugae* adults arboreal habitats in western Oregon (Lamana and were obtained from colonies at Clemson Miller 1996) and West Virginia (Brown and Miller University (Clemson, South Carolina) and the 1998). The mechanisms of replacement are not University of Tennessee (Knoxville, Tennessee). well understood, but direct or indirect competitive *H. axyridis* adults were collected locally in interactions among species utilizing the same southwestern Virginia (Jefferson National Forest, resource at the same time may lead to reductions Virginia). Laboratory populations of *L. nigrinus* in predator diversity and decrease the efficacy of and *H. axyridis* had been supplemented with wild biological control. These interactions can change beetles the previous year, while *S. tsugae* were community structure and ultimately result in the descended from a population that had been escape of the prey population (Rosenheim et al. consecutively reared for several generations. 1993), but few studies of competition among Predator rearing was in accordance with predators have been conducted in forest previously established methods for *L. nigrinus* ecosystems.

(10C, 12:12 L:D, 75% RH) (Lamb et al. 2005b) and the coccinellids (25C, 16:8 L:D, 45% RH) Beginning in 2002, studies were undertaken to

(Palmer and Sheppard 2002; Matsuka and determine the nature and effects of intraspecific Nijjima 1985). Food adaptation was not a major and interspecific competition among these species concern for *L. nigrinus* and *S. tsugae* as they are so that the establishment and resilience of newly considered specialists on this prey. The generalist introduced and existing populations are predator, *H. axyridis*, was provided with a small minimally affected. Both laboratory and field amount of honey and wheat mixture during the studies by Flowers et al. 2005; 2006 suggest that holding phase, prior to the experiments, to serve *L. nigrinus*, *S. tsugae* and *H. axyridis* are as a nutritional supplement. For the coccinellid compatible with one another; however, species, conditions were gradually stepped down competitive interference was shown to occur from 20 to 12C and from 16:8 to 12:12 L:D over a among conspecifics. Compatibility was assessed 2±3 wk period to preycondition them to the by examining direct impact on the survival, conditions used in the spring evaluation. At the feeding and reproduction of one another when conclusion of the spring trials, conditions were enclosed together in shortyterm laboratory assays

gradually increased from 12 to 22C and from and longeryterm field enclosures. However, these 12:12 to 16:8 L:D over the same time interval to studies did not examine more subtle, indirect preycondition predators for summer trials. All effects that may have occurred, especially in species were held in 2.2 L plastic containers lined regard to behavioral changes, which can with moistened filter paper and ventilated with negatively impact predator effectiveness. fine polyester mesh (PeCap, Sefar, Currently, there exist no detailed studies of www.sefar.com). Each container held 15 adults, in predator behavior and daily activity patterns of a sex ratio of 2F:1M, and 5±7 *T. canadensis* these species with *A. tsugae*, and what type of branch clippings heavily infested with *A. tsugae*. behavioral changes may occur in the presence of Containers were maintained in an environmental additional predators is unknown. This study was chamber (Model Iy36, PercivalScientific, undertaken to determine the daily activity www.percivalyscientific.com). Adult predators
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Figure 1. Experimental design for video studies. Each assay consisted of a 5 x 2.5 cm Petri dish lined with moistened filter paper, and held two 5 cm clippings of eastern hemlock, *Tsuga canadensis*, infested with 10 *Adelgus tsugae*. A digital video camera was mounted on a camera stand 5±10 cm above the assay and used for video capture (A). All intermittent video recording was controlled using EvoCam 3.5 software and video segments were visualized and stored on an Apple iMac G4 computer (B).

were transferred from holding containers to assays 24 h before recording. All predator females were mature, of approximately the same age (12±24 wk), and were selected randomly from preyconditioning containers. Morphological characters were used to separate the sex of *S. tsugae* (Sasaji and McClure 1997), and *H. axyridis* (Gordon 1985), while monitoring oviposition over 72 h was used for *L. nigrinus*.

Experimental design of video studies Predator behavior and daily activity patterns of adult females of each species were documented in the laboratory using intermittent digital video recording. Predator arenas consisted of 5 x 2.5 cm Petri dishes (Fisherbrand, FisheryScientific, www.fishersci.com) lined with moistened filter paper. The diameter of the assay represented 50±100X the body widths of these predators. Experiments were conducted in an environmentally controlled room using two evaluation periods based on temperature and RH averages obtained by data loggers (Hobo, Onset Computer, www.onsetcomp.com) placed in hemlock stands in southwestern Virginia. The evaluations were termed 1) spring: 12 2°C, 12:12 L:D, 50±75% RH, and 2) summer: 22 2°C, 16:8 L:D, 65±85% RH. These conditions cover the approximate duration when *A. tsugae* sistens and progrediens adults with ovisacs are present, respectively (McClure 1989). Each assay contained two 5 cm hemlock clippings, which were infested with 10 *A. tsugae* sistens (spring) or progrediens (summer) adults with ovisacs. *A. tsugae* has a clumped distribution at low to moderate

densities. These patches have much higher densities than surrounding areas, and the assay was designed to represent such an area. Special attention was paid to prey quantity and quality. Prey quantity was determined from previous laboratory studies (Flowers et al. 2005), such that predators were allowed to feed *ad libitum* during the evaluation. High prey quality was also maintained by microscopically examining areas adjacent to branch clippings used in the trials, to insure that adults were alive and possessed ovisacs with a similar number of eggs. Each predator species was evaluated singly, and combined with one additional conspecific or heterospecific predator in all possible combinations. All species were included in the spring evaluation; however, *L. nigrinus* was excluded from the summer evaluation due to its aestivation period (ZilahiyBalogh et al. 2003c).

Video capture was done using a onechip digital camcorder (PVyGS35, Panasonic, Knoxville, TN) mounted on a camera stand 5±10 cm directly above the arena (Figure 1). The camcorder had a 30X optical lens and 1000X digital zoom. Automatic settings were generally used to control camcorder exposure, gain, focus and whitebalance levels; however, manual adjustments were made as necessary to improve overall video quality. A video output cable (FireWire IEEE 1394, Apple Computer, Cupertino, CA) was used to link the camcorder directly to a computer (iMac G4, Apple Computer, Cupertino, CA) for direct transfer of all video segments. Digital video software (EvoCam 3.5, Evological, www.evological.com) was used to visualize the arena, control videocapture and

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compress video segments prior to storage on the only a single overhead view of the arena was hard drive and eventual archiving onto compact available. Therefore, oviposition events were disc. Oneyminute video recordings were captured determined indirectly using microscopy every 15 min over 24 h for a total of 96 examination of branch clippings at the conclusion observations per day. Each treatment was of each assay. For all species, each predator egg prepared in duplicate, with one assay recorded found was designated as a single oviposition event during the day and one at night. Video segments for that species during that time period. Predator were compressed to MPEG format and reviewed eggs were distinguished using morphological using QuickTime 7.0 software (Apple

characteristics of *L. nigrinus* (ZilahiyBalogh et al. Computer). Two light sources with white 2003c), *S. tsugae* (Sasaji and McClure 1997) and translucent diffusing filters were positioned 0.5±1 *H. axyridis* (Gordon 1985). Resting behavior was m on each side of the arena to illuminate the designated as predators maintaining a stationary arena adequately for filming. Day recordings used position on the branch or assay surface. Behavior ambient light supplemented by 25W softywhite descriptions for each species were based on a light bulbs (Philips), while night recordings used review of 15 randomly selected video segments of 25W red light bulbs (Philips). Pilot studies were each behavior category from singleypredator assay completed to determine optimal assay conditions recordings. Predator daily activity patterns were for filming and to assure that predator behavior determined by summing the number of scored was not significantly altered in response to events for each behavior category over each time experimental conditions. An electronic data period (day/night). Video segments in which logger (Hobo, Onset Computer) was placed next predator behavior could not be clearly to the assay to monitor environmental conditions. distinguished or consisted of multiple behaviors Six replications of each treatment were were excluded from the analyses. completed, with three occurring during the day and three at night.

Predator effects on temporal and spatial Predator behavior and daily activity The video recordings were reviewed and scored for behavior exhibited and relative location of each predator. Each recording was assigned to one of five general behavior categories: 1) Extensive searching, 2) Intensive searching, 3) Feeding, 4) Oviposition, or 5) Resting. For all species, the duration of each behavior was consistently greater than the 1 min recording interval, so each video segment could be assigned to a single behavioral category. Searching behavior was divided into extensive search, distinguished by rapid movement over a large area of the assay or branch surface, and intensive search, distinguished by slower movements over a small area of the branch surface. Predator placement of the mouthparts, head or body within *A. tsugae* ovisacs was used to designate feeding behavior. This was often accompanied by visual evidence of disrupted woolly filaments and the presence of adelgid hemolymph on the ovisac
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patterns In pairedypredator assays, behavior was scored for one marked predator, chosen at random, in conspecific combinations and for each species in heterospecific combinations. The impacts of intray and interspecific competition on predator daily activity patterns were assessed by comparison of the results of singly and pairedypredator trials for each species. In the absence of significant competitive interference, similarity among the counts of each behavior category would be expected among the treatments. A significant shift in the number of behavior events was used to indicate predator interference. For spatial analyses of predator pairs, the approximate separation (0 ± 5 cm) between predators was assessed at the start of each recording, and distances were averaged for each treatment. Separation distances were measured from the center of the body of each predator to account for size differences among these species. surface. Close inspection of *A. tsugae* ovisacs, by *L. nigrinus*, or of branch and needle substrates by

Statistical analysis *S. tsugae* and *H. axyridis*, followed by extension

Predator behavior analyses as well as changes in of the posterior abdominal segments (functional temporal and spatial activity patterns were ovipositor) was used to designate oviposition examined separately for the spring and summer behavior. For *L. nigrinus*, egg deposition could evaluation using a mixed model (proc mixed;SAS not be directly observed because of placement Institute 2001, www.sas.com). The model inside the adelgid ovisac. Similarly, egg deposition incorporated both fixed factors and a random by the coccinellid species on branch or needle covariate, as behavior was documented for substrates, was difficult to observe directly, as individual predators and required a hierarchical

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analysis since the individual served as a block for

with *A. tsugae* ovisacs, after which this search the behaviors. Singleypredator assay comparisons pattern would cease and an apparent closer were analyzed separately by behavior category

inspection of prey would occur. For *H. axyridis*, and the model included predator species and time intensive searching appeared to be less systematic (day/night) as fixed categorical variables and than that of the specialist predators. Females predator species by replication as the random would usually move from an extensive searching covariate. Paired predator temporal behavioral pattern or a resting location directly to a prey in analyses were examined separately by predator close proximity. Prey selection was rapid and species and behavior category, while feeding often began immediately. paired predator spatial analyses were examined separately by behavior category only. The model

Feeding by adult female predators was similar in in each case included predator species form for all species. The mandibles were used to combination and time as fixed categorical disrupt the ovisac filaments to obtain access to variables and predator species combination by adult and immature *A. tsugae*. Specialist replication as the random covariate. Treatment predators would usually feed on some of the outer means were analyzed using two-way analysis of eggs of the adelgid ovisac, then advance further variance followed by Tukey's HSD to separate into the ovisac until only the posterior abdomen treatment means (Zar 1998). Behavior event was visible, causing a low level of disruption counts were log₁₀ transformed to achieve (Video). When attacking adelgid adults, specialist normality and equality of variances, and all predators would often make a single bite on the results were evaluated for significance at $P = 0.05$.

thorax or abdomen, releasing hemolymph that was ingested by the adult, and resulting in a collapsed *A. tsugae* exoskeleton. In contrast, *H. axyridis* would greatly disrupt adelgid ovisacs to

Results
Predator
behavior

feed on adults and immatures, often consuming a large portion of the eggs and adult exoskeleton by qualitatively for each species. Extensive searching chewing (Video). Feeding by the specialists behavior was similar in form for all species. It occurred more often and for shorter durations occurred primarily on the upper and lower assay than *H. axyridis*. surfaces and consisted of successive rapid movements covering a large area. The behavior Predator oviposition and resting behavior for was sometimes followed by relocation onto these species was similar in form and distinct in branch surfaces, and a rapid shift to intensive location. For *L. nigrinus*, there were tapping and searching behavior occurred. In other cases, the repeated insertions of the posterior abdominal behavior would occur over longer periods of time, segments into the adelgid ovisac before egg and may represent predator agitation and an deposition (Video). Lateral movements of the legs attempt to disperse from the assay. Intensive and abdomen helped to drive these segments searching by *L. nigrinus* consisted of successive deeper into the ovisac. For this species, eggs were inspection of *A. tsugae* ovisacs using a series of usually placed singly, but 2±3 eggs would short linear movements from one prey location to sometimes be placed at the same location once all another (Video). During prey inspection, there available ovisacs contained eggs. Oviposition by *S.* was often tapping of the antennae and abdomen *tsugae* and *H. axyridis* was very low overall, and on the surface of the adelgid ovisac. After difficult to document in many cases due to surveying numerous prey items, adult females experimental limitations. It appeared that the would remain at a single location for additional these coccinellid species would first inspect the inspection, and this was often followed by feeding foliage or branch surface, followed by extension of or oviposition behavior. Intensive searching by *S.* the posterior abdominal segments, which would *tsugae* consisted of a series of systematic probe host substrates before oviposition. For *S.* movements along the needle and branch surfaces. *tsugae*, eggs were usually placed singly in close In most cases, one side of the branch clipping proximity to adelgid ovisacs on hemlock bud would be surveyed at a time by horizontal scales or in bark crevices with the eggs slightly movements along each side of several needles in

exposed. In contrast, oviposition by *H. axyridis* succession (Video). This often resulted in contact was in groups of 5 ± 15 eggs placed on needle

Video. This video can be accessed at the following URI: <http://digital.library.wisc.edu/1793/11389>

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Figure 2. Mean number (SE) of behavior events per day for adult female *Laricobius nigrinus*, *Sasajiscymnus tsugae* and *Harmonia axyridis* grouped into five behavior categories (Extensive Searching, Intensive Searching, Feeding, Resting, Oviposition) during (A) spring (122°C, 12:12 L:D, 50±75% RH) and (B) summer (222°C, 16:8 L:D, 65±85% RH) evaluations in single predator assays (n 6). One minute video segments were captured every 15 min over 24 h. Mean was significantly different at $P < 0.05$, ns no significant difference among means for the behavior.

surfaces in close proximity to adelgid ovisacs. Lastly, resting behavior by *L. nigrinus* and *S. tsugae* usually occurred at concealed locations on the branch surface in close proximity to *A. tsugae*.

Predators were often located at the junction of the stem and a branchlet or between two needles at the base of branchlets. In contrast, resting by *H. axyridis* occurred at more exposed locations on

Figure 3. Mean number (SE) of behavior events per day for adult female (A) *Laricobius nigrinus* (*Ln*), (B) *Sasajiscymnus tsugae* (*St*) and (C) *Harmonia axyridis* (*Ha*) grouped into five behavior categories (Extensive Searching, Intensive Searching, Feeding, Resting, Oviposition) during the spring evaluation (12.2°C, 12:12 L:D, 50±75% RH) in paired predator assays (n 6). Each species was tested alone and in combination with one conspecific or heterospecific predator. One minute video segments were captured every 15 min over 24 h. Analyses were done separately for each behavior. Mean was significantly different at $P < 0.05$, ns no significant difference among means for the behavior. [continued on next page]

branch and arena substrates often at greater distances from *A. tsugae*.

Predator daily activity In single predator behavior

comparisons, there was a significant interaction between predator species and behavior category in spring (F 5.34; df 8,54; P 0.0001) and summer (F 4.63; df 4,36; P 0.0001), so each behavior category was analyzed separately. For the behavior

Figure 3. [from previous page] Each species was tested alone and in combination with one conspecific or heterospecific predator. One minute video segments were captured every 15 min over 24 h. Analyses were done separately for each behavior. Mean was significantly different at P 0.05, ns no significant difference among means for the behavior.

category analyses, there were no significant interactions between the variables, so each factor was evaluated across the other variable levels.

During the spring, there were significant differences by predator species for extensive searching, intensive searching, feeding, oviposition and resting (Figure 2A, Table 1). Extensive searching and resting behaviors were greater in the coccinellid species than in *L. nigrinus*, which showed greater intensive searching behavior and oviposition than the coccinellid species.

Feeding behavior by *L. nigrinus* and *H. axyridis* was similar in the spring, and counts for each species were greater than for *S. tsugae*. Overall, the distribution of events across behavior categories was more even for *L. nigrinus*, while the coccinellid behaviors were skewed toward resting.

During the summer evaluation, significant differences also occurred by predator species for intensive searching and feeding, while extensive searching, oviposition and resting were similar (Figure 2B, Table 1). Intensive searching behavior was greater for *S.*

tsugae than for *H. axyridis*. The latter had a greater number of feeding events than *S. tsugae*. During the summer evaluation, behavior for these species appeared to be more evenly distributed across the

categories than in the spring.

For the second factor, time (day/night), there were no significant differences in the spring for

Table 1. Daily activity comparisons of single predators by species and time (day/night).

	Spring	Summer	Predator	Species	F	df	P	F	P	F	P									
Intensive searching	6.12	2.6	0.036	5.56	1,4	0.047	Feeding	10.73	2,6	0.011	50.13	1,4	0.002	Oviposition	4.58	2,6	0.04	1.18	1,4	0.339
Resting	16.78	2,6	0.004	3.32	1,4	0.142														
Time	F	df	P	F	df	P	Extensive searching	0.28	1,6	0.617	3.44	1,4	0.137	Intensive searching	3.19	1,6	0.125	9.14	1,4	0.039
Feeding	0.49	1,6	0.509	109.21	1,4	0.0005	Oviposition	0.08	1,6	0.936	0.58	1,4	0.489							
							Resting	0.1	1,6	0.909	87.91	1,4	0.0007							

Figure 4. Mean number (SE) of behavior events per day for adult female (A) *Sasajiscymnus tsugae* (*St*) and (B) *Harmonia axyridis* (*Ha*) grouped into five behavior categories (Extensive Searching, Intensive Searching, Feeding, Resting, Oviposition) during the summer evaluation (22.2°C, 16:8 L:D, 65±85% RH) in paired predator assays (n 6). Each species was tested alone and in combination with one conspecific or heterospecific predator. One minute video segments were captured every 15 min over 24 h. Analyses were done separately for each behavior. Mean was significantly different at $P < 0.05$, ns no significant difference among means for the behavior.

Figure 5. Mean separation distance (SE) between adult female *Laricobius nigrinus* (*Ln*), *Sasajiscymnus tsugae* (*St*) and *Harmonia axyridis* (*Ha*) in the (A) spring (122°C, 12:12 L:D, 50±75% RH) and (B) summer (222°C, 16:8 L:D and 65±85% RH) evaluations for the paired predator assays (n 6). Each species was tested in combination with one conspecific or heterospecific predator. One minute video segments were captured every 15 min over 24 h. Means with the same letter were not significantly different at P 0.05.

2). In contrast, *H. axyridis* again showed significant

differences by predator species combination for extensive searching, feeding, oviposition and resting,

while intensive searching was similar (Figure 4B, Table 2). Consistent with the spring evaluation, the conspecific combination led to increased extensive searching as

well as reduced feeding, oviposition, and resting.

By time (day/night), the spring evaluation revealed significant differences for *L. nigrinus* in

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intensive searching, feeding and resting, while involves two patterns of movement. Extensive extensive searching and oviposition were similar searching uses rapid linear movements between (Table 3). There was greater intensive searching prey patches, while intensive searching includes behavior at night, while feeding and resting were slower and more directed movements that are greater during the day. For *S. tsugae*, no often induced by the perception of prey cues (Bell significant differences by time were observed for 1990). In predatory beetles, changes to search any of the behaviors in the spring (Table 3). The behavior often occur in response to prey detection results for *H. axyridis* revealed significant and capture, which results in a switch from differences by time for extensive searching, extensive to intensive, or local areayconcentrated, intensive searching, feeding and resting, while searching (Nakamuta 1982; 1985). This varies only oviposition was similar (Table 3). Extensive based on the ability of the predators to detect and intensive searching, as well as feeding, were preyyspecific cues, and appears to be greater during the day, while resting occurred advantageous for predators whose prey display a more at night.

clumped distribution. Previous studies of *H. axyridis* indicate that this species uses vision and In the summer, *S. tsugae* showed significant olfaction to find prey (Obata 1997; Harmon et al. differences by time for extensive and intensive 1998; Han and Chen 2002), and are consistent searching, as well as feeding and resting, while with the observations presented here with A. only oviposition was similar (Table 3). Results tsugae. Adults of *H. axyridis*, unlike the specialist were similar for *H. axyridis*, with extensive predators, did not appear to follow any type of searching, intensive searching, feeding and systematic search pattern to find prey, but resting significantly different by time, while consistently demonstrated the ability to move oviposition was similar (Table 3). For both directly to prey locations under both day and coccinellid species, extensive and intensive night conditions. While specific cues were not searching, as well as feeding, was greater during directly tested in this study, the behavioral the day, while resting events were greater at night.

observations are suggestive of their use. Olfaction may also be used in prey finding by *L. nigrinus*, **Predator effects on spatial patterns** Spatial activity comparisons in the pairedpredator trials showed no significant interactions between predator species combination and time during spring (F 1.67; df 5,12; P 0.218) or summer (F 0.46; df 2,6; P 0.651), and each factor was evaluated across all other variable levels. There were significant differences by predator species combination during both spring (F 7.46; df 5,12; P 0.002) (Figure 5A) and summer (F 23.27; df 2,6; P 0.001) (Figure 5B). In the spring, the conspecific

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which possess antennae with several olfactory receptors (Broeckling and Salom 2003), and may be able to detect *A. tsugae* volatiles. Field studies indicate that *L. nigrinus* can locate *A. tsugae* at low population densities in its native range (Mausel 2005), suggesting that it may have longyrange prey perception ability. In contrast, *S. tsugae* has a less sophisticated antennal morphology, with few olfactory receptors (Broeckling and Salom 2003), and thus may rely more on vision and direct contact cues to locate prey, as was observed in this study. pairing of *H. axyridis* showed the greatest average separation distance, followed by *L. nigrinus* conspecifics and all heterospecific

pairings, which were similar. During the summer evaluation, *H. axyridis* conspecifics once again maintained the greatest spatial separation, followed by the heterospecific pairing. The conspecific pairing of *S. tsugae* were found in closest proximity during each evaluation. Predator separation distances were not significantly different by time during the spring (F 4.19; df 2,12; P 0.068) or summer (F 0.52; df 1,6; P 0.497) evaluation. The differences in intensive searching behavior in these species are likely related to predator feeding and reproductive biology. For *L. nigrinus* and *S. tsugae*, greater prey evaluation may be a product of their close association with *A. tsugae* (ZilahiyBalogh et al. 2002; Cheah and McClure 1998). Eggs of *L. nigrinus* are laid directly within adelgid ovisacs, and thus, it would be advantageous for females to insure that each ovisac is of sufficient size or quality for the successful development of progeny. Similarly, rearing studies indicate that *S. tsugae* is sensitive

Discussion

to prey quality (Palmer and Sheppard 2002), which may explain its more thorough prey Within habitat host searching by aphidophagous predators does not usually occur randomly, but evaluation once *A. tsugae* was located. In contrast, *H. axyridis* may not be adapted to detect Journal of Insect Science | www.insectscience.org ISSN: 1536y2442

qualitative differences of *A. tsugae*, and intensive visibility of *H. axyridis* eggs appears to be related searching in this species appears to be directed to their inherent chemical deterrents, which toward areas of high prey density that are within provide protection against interspecific predation close proximity. These results are consistent with (Ayer and Brown 1977; Hemptinne et al. 2000; previously described areayconcentrated searching Agarwala and Yasuda 2001). In addition, the behavior for *Coccinella septempunctata* L., a bright coloration and clumped distribution of eggs closely related coccinellid predator (Nakamuta may serve to warn heterospecifics, and provide 1985; 1987) and for *H. axyridis* with aphid prey in increased detection and food availability to Japan (Obata 1986; Osawa 2000). While the conspecifics. When prey is of low quantity or former species used primarily visual cues, a quality, cannibalism has been shown to increase combination of visual and olfactory cues appears progeny growth and survival (Wagner et al. 1999; to be used by *H. axyridis* to guide movements to Snyder et al. 2000; Michaud and Grant 2004). prey.

Variations in resting behavior followed a similar Variations in feeding and oviposition behaviors pattern, with *L. nigrinus* and *S. tsugae* being are also consistent with what is known regarding located in more concealed locations and *H. predator* foraging and reproductive biology. *axyridis* maintaining greater apparency. Feeding and oviposition behaviors by *L. nigrinus* Generalist predators such as *H. axyridis* often and *S. tsugae* were consistent with what has been engage in intraguild predation when prey described previously for another specialist becomes scarce (Yasuda and Shinya 1997; Burgio predator of *A. tsugae* (Lu et al. 2002). et al. 2002; 2005); therefore, more concealed Microscopic examinations of assays confirmed resting locations may provide additional that feeding events by *L. nigrinus* and *S. tsugae* protection for the specialist species. Greater were often limited in extent. Feeding that is less apparency for *H. axyridis* would not be disruptive to the ovisac may be advantageous for detrimental to their survival given their chemical *L. nigrinus*, as it may provide increased ecology and warning coloration, and may serve to protection for progeny that develop within the increase mateyfinding (Majerus 1994). adelgid ovisac. In contrast, feeding by *H. axyridis* was generally more extensive at each site and

Daily predator activity for these species can be resulted in a greater disruption to ovisac generally classified as continuous, in which filaments. This in turn may serve to better shorter searching, feeding and oviposition events facilitate entry by progeny that emerge from eggs are punctuated by longer periods of rest. In the placed on host substrates in close proximity. The

spring, increased activity and a more even ovipositional behavior observed for *L. nigrinus* behavior distribution by *L. nigrinus* was likely may serve to provide an additional assessment of due to this species being highly coldadapted prey quality or potentially assist in the detection (Lamb et al. 2005a; ZilahiyBalogh et al. 2003b). of conspecific chemical markers or conspecific Behavior in these species is influenced by eggs. This could allow *L. nigrinus* to reduce temperature (Cheah and McClure 1998; Stathas et intraspecific interactions among progeny, which is al. 2001; ZilahiyBalogh et al. 2002); the detrimental to larval survival. A similar type of developmental threshold of *L. nigrinus*, which is assessment, of branch and needle substrates, also lower than *S. tsugae* or *H. axyridis* by 5.8 and occurred for the coccinellid species before 7.5C, respectively (Cheah and McClure 1998; oviposition. These behaviors may serve to Lamana and Miller 1998; ZilahiyBalogh et al. discriminate locations of better attachment or 2003c), allowed this species to maintain greater protection of eggs, and could lead to increased activity levels under colder conditions. Consistent progeny survivorship.

with this result, *S. tsugae* and *H. axyridis* appeared to have a more even behavioral Predator oviposition locations may be related to

distribution at higher temperatures in the reducing interspecific competition, as eggs of *L.* summer evaluation. However, even when *nigrinus* and *S. tsugae* are vulnerable to temperatures were similar to optimal rearing predation by all three species (Flowers et al. 2005; conditions for the coccinellid species, oviposition 2006). Thus, placement at more concealed may still have been limited due to experimental locations lowers the risk of predation or provides constraints such as insufficient prey quality and a higher degree of buffering against host health for *S. tsugae* (Palmer and Sheppard environmental conditions. In contrast, the 2002) and nutrient limitations for *H. axyridis*

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(Dixon 2000; Soares et al. 2004). In addition, the

Each species displayed distinct temporal patterns design may also have limited the dispersal ability in which behaviors occurred more frequently of these species to potentially more favorable during day or night. For *L. nigrinus*, greater microhabitats.

intensive searching at night and resting during the day may indicate this species to be more

Conspecific predators exerted influence on the behavior patterns of *L. nigrinus* and *H. axyridis*, but not of *S. tsugae*. In the spring, the addition of *L. nigrinus* conspecifics increased searching behaviors and decreased resting, while feeding and oviposition remained similar. This response is most likely related to prey selectivity, as females had to spend more time locating acceptable oviposition sites. Predator effectiveness, in terms of energy expenditure, would therefore be reduced, as *L. nigrinus* dedicated more time and resources to searching behaviors, which do not serve to directly impact adelgid prey. If behaviors in this species were altered in response to passive chemical cues associated with conspecific contact of prey or branch substrates, we would expect feeding and oviposition rates to be affected also; however, these responses were not affected. The results also indicate that females of this species may be adapted to detect the presence of eggs or ovipositionydeterring chemical cues of conspecifics. The wideyranging effects of conspecifics on *H. axyridis* behavior are consistent with previous studies of the chemical ecology of this predator. Intraspecific interactions

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nocturnal than the other two predators. Increased behavior at this time is possible because of adaptation to cold (ZilahiyBalogh et al. 2003a), and may serve to decrease susceptibility to predators or reduce co occurrence with other species. Searching behavior during this time also suggests that it may rely more on olfaction than vision to locate and evaluate prey. In contrast, *S. tsugae* and *H. axyridis* were more active during the day, consistent with previous observations of other coccinellid predators (Nakamuta 1987; Majerus 1994) and *H. axyridis* (Obata and Johki 1990). Greater searching and feeding activity during the day may be related in some degree to increased

daytime temperatures, but more likely, this may have resulted from their using visual cues to locate *A. tsugae*. To optimize finding prey and conserve energy and resources, search activity in these species may be reduced at night due to a lower success of prey detection in total darkness. Oviposition by the coccinellids would likely have been statistically higher during the day as well due to similar factors, but overall low levels of reproduction make these inferences difficult.

in this species are regulated by both passive substrate marking using fecal cues (Agarwala et al. 2003) and actively deposited oviposition-detering pheromones (Yasuda et al. 2000). Given the temporary nature of many prey populations and its ability to cannibalize (Osawa 1993; Hironori and Katsuhiko 1997; Burgio et al. 2002; 2005), selective pressure would favor avoiding or reducing activity in areas already occupied by conspecifics. This was consistent with the results observed in these trials where extensive searching was increased, and feeding and oviposition were reduced. Conspecific effects for *S. tsugae* may have been masked by the predominance of resting behavior in these trials, as previous studies indicated that this species has the ability to cannibalize (McClure 1995), and its behavior may therefore be regulated by similar mechanisms. In contrast, predator temporal activity patterns were not significantly altered by heterospecifics, indicating that these species may be compatible in this system and suggesting that these predators may not be adapted to detect the chemical cues of one another.

Relative to assay size, all predator pairings appeared to maintain a high degree of spatial separation. While it is difficult to assess the responses due to the limited size and architecture of the assay, this suggests that avoidance behaviors may occur in these species. Distances were greatest between conspecifics of *H. axyridis* and this may again be in response to chemical cues, as has been documented in spatial studies of similar predator species (Grostal and Dicke 1999). The shorter separation distances for *L. nigrinus* and *S. tsugae* conspecifics, by comparison, may indicate these species have a less sophisticated chemical ecology. For *S. tsugae*, distance measures were influenced by the predominance of resting behavior, during which conspecifics were found in close proximity to one another. However, separation between *S. tsugae* conspecifics during searching and feeding events was similar to that of the other species. Heterospecific pairings also exhibited high spatial separation within the context of these assays. Overall, interspecific effects on spatial patterns appeared to be in response to tactile (direct contact), rather than

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chemical cues. Video evidence consistently

In conclusion, additional examinations of these showed that predator disturbance and relocation predators under field conditions are necessary to was high in response to direct contact, corroborate descriptions of predator behavior and particularly by the specialists when contacted by daily activity. However, activity patterns for *H. H. axyridis*. Avoidance behaviors and changes in *axyridis*, documented in field studies in Japan spatial patterns in response to heterospecifics (Obata and Johki 1990), were very similar to have been shown to occur in other aphidophagous those found for this species using the laboratory predator guilds (Musser and Shelton 2003; Sato techniques presented here. These behavioral et al. 2005), and may regulate spatial analyses support our previous studies that relationships in this system as well.

indicate these species to be compatible within a biological control program for *A. tsugae*. Inferences based on these observations are

Temporal activity patterns were not highly limited due to the experimental design and coordinated, and predator avoidance responses duration, which may have exerted a great deal of appear to be such that these species will be able to influence on the behavior of these species. The maintain a high degree of spatial separation evaluation of extensive search behavior was under more natural conditions. Therefore, we perhaps most limited, as predators were recommend using multiple predator prohibited from leaving the arena; however, the combinations of the specialist predators, over movement pattern exhibited within the arena was single species when implementing biological suggestive of extensive search behavior. control for *A. tsugae*. In addition, implementing Consanguinity should also be considered, as low density predator releases may reduce the laboratory populations may not accurately

potential negative effects associated with represent the behavioral patterns that occur intraspecific interference and lead to improved within the larger population. However, L. predator effectiveness under field conditions. *nigrinus* and *H. axyridis* colonies were regularly supplemented with wild beetles collected in the field. For *S. tsugae*, adults were descended from a

Acknowledgements

limited, original collection and have been

The authors thank Tom McAvoy, Ashley Lamb, consecutively reared for several generations, and David Mausel, Brian Eisenback and cooperators thus may have reduced behavioral variability. at Clemson University and The University of Predators have also found to display a high degree TennesseeKnoxville for technical assistance. We of plasticity in individual behaviors, and previous would also like to thank Drs. Douglas Pfeiffer and studies indicate that searching patterns can Michael Montgomery for reviewing earlier change in response to prey type, and that behavior versions of this manuscript. Funding for this may be conditioned in some beetle species with research was provided by grant no. continuous exposure to a single prey (Ettifouri 05yDGy11083150y050 from the USDA Forest and Ferran 1993). Also, the techniques necessary Service, Special Technology Development to study predator behavior in detail do not allow Program. for the immigration and emigration that may occur on a larger scale in response to prey abundance or intraspecific and interspecific predator cues. Video recordings of predator behavior were difficult to obtain using a single camcorder due to the architecture of the hemlock

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