

Host Range Specificity of *Scymnus camptodromus* (Coleoptera: Coccinellidae), A Predator of Hemlock Woolly Adelgid (Hemiptera: Adelgidae)

Samita Limbu,¹ Katie Cassidy,¹ Melody Keena,² Patrick Tobin,³ and Kelli Hoover^{1,4}

¹Department of Entomology and Center for Chemical Ecology, Penn State University, 501 ASI Bldg., University Park, PA 16802 (szl175@psu.edu; kem5052@gmail.com; kxh25@psu.edu), ²USDA Forest Service, Northern Research Station, 51 Mill Pond, Hamden, CT 06514 (mkeena@fs.fed.us), ³School of Environmental and Forest Sciences, University of Washington, 123 Anderson Hall, Seattle, WA 98195 (pctobin@uw.edu), and ⁴Corresponding author, e-mail: kxh25@psu.edu

Received 17 July 2015; Accepted 2 November 2015

Abstract

Scymnus (Neopullus) camptodromus Yu and Liu (Coleoptera: Coccinellidae) was brought to the United States from China as a potential biological control agent for hemlock woolly adelgid (*Adelges tsugae* Annand) (Hemiptera: Adelgidae). *Scymnus camptodromus* phenology is closely synchronized with that of *A. tsugae* and has several characteristics of a promising biological control agent. As a prerequisite to field release, *S. camptodromus* was evaluated for potential nontarget impacts. In host range studies, the predator was given the choice of sympatric adelgid and nonadelgid prey items. Nontarget testing showed that *S. camptodromus* will feed to some degree on other adelgid species, but highly prefers *A. tsugae*. We also evaluated larval development of *S. camptodromus* on pine bark adelgid (*Pineus strobi* (Hartig)) (Hemiptera: Adelgidae) and larch adelgid (*Adelges laricis* Vallot) (Hemiptera: Adelgidae); a small proportion of predator larvae was able to develop to adulthood on *P. strobi* or *A. laricis* alone. *Scymnus camptodromus* showed no interest in feeding on woolly alder aphid (*Paraproctiphilus tessellatus* Fitch) (Hemiptera: Aphididae) or woolly apple aphid (*Eriosoma lanigerum* (Hausmann)) (Hemiptera: Aphididae), and minimal interest in cotton aphid (*Aphis gossypii* Glover) (Hemiptera: Aphididae) in choice and no-choice experiments. *Scymnus camptodromus* females did not oviposit on any host material other than *A. tsugae*-infested hemlock. Under the circumstances of the study, *S. camptodromus* appears to be a specific predator of *A. tsugae*, with minimal risk to nontarget species. Although the predator can develop on *P. strobi*, the likelihood that *S. camptodromus* would oviposit on pine hosts of this adelgid is small.

Key words: host range, *Scymnus camptodromus*, biological control, nontarget effect, invasive species

Hemlock woolly adelgid (*Adelges tsugae* Annand) (Hemiptera: Adelgidae) is a major pest of eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelman, in the eastern United States, causing marked tree decline and mortality (McClure 1991). It was originally introduced into the eastern United States from southern Japan (Havill et al. 2006). *Adelges tsugae* is not considered a pest in its native range due to the presence of a complex of natural enemies and naturally resistant hemlocks (Cheah and McClure 2000, Cheah et al. 2004). Although *A. tsugae* is endemic to the western United States, the biotype there is genetically distinct from the introduced *A. tsugae* in the eastern United States that causes severe injury and extensive tree decline (Havill et al. 2006).

Adelges tsugae has spread rapidly since its introduction; there are no known parasitoids, insufficient generalist native predators, and an abundance of susceptible hemlock stands in the eastern United States (Montgomery and Lyon 1996, Wallace and Hain 2000, Hakeem et al. 2013, Havill et al. 2014). This pest is currently

distributed over 19 states in the eastern United States, encompassing ~40% of the *T. canadensis* range and 100% of the *T. caroliniana* range (U.S. Department of Agriculture–Forest Service [USDA-FS] 2012, Preisser et al. 2014). The rate of spread has been estimated at about 15 km per year in the South and 8 km per year in the North (Evans and Gregoire 2007). Proliferation of *A. tsugae* is believed to be more rapid in the South due to the warmer climate, which allows prolonged feeding and reduced winter mortality of adelgids, resulting in faster hemlock decline (Parker et al. 1999, Skinner et al. 2003, Ford et al. 2012).

Evaluation of classical biological control agents for managing *A. tsugae* populations has been progressing in earnest for several years (Butin et al. 2003, 2004; Vieira et al. 2011; Story et al. 2012; Jones et al. 2014; Limbu et al. 2015) and is considered the most promising approach for forest settings (Onken and Reardon 2011). The aim is to build a community of predators that collectively keeps the pest population below damaging levels.

Natural enemies introduced in the eastern United States for control of *A. tsugae* include several predatory insect species collected from the native range of *A. tsugae* worldwide (Cheah and McClure 1998, Mausel et al. 2010, Montgomery and Keena 2011, Montgomery et al. 2011, Hakeem et al. 2013). Two of those predatory species, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) and *Sasajiscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae), have been released, recovered, and were found coexisting in the areas of release (Hakeem et al. 2011). However, studies also suggest that predation is somewhat temperature dependent; for example, some predators are more active during cooler spring temperatures and some during warmer summer temperatures (Flowers et al. 2006). Therefore, a complex of natural enemies will likely be required to significantly reduce *A. tsugae* populations in different seasons and geographic locations.

Exploration for natural enemies of *A. tsugae* included a collection of *Scymnus* (*Neopullus*) *camptodromus* Yu and Liu (Coleoptera: Coccinellidae) from the Sichuan and Yunnan provinces in the southwestern China (Montgomery and Keena 2011). *Scymnus camptodromus* is an efficient predator in its native ecosystem; the adults feed on all life stages of *A. tsugae* throughout their development (Cheah et al. 2004). The larvae also feed on multiple life stages, although they feed most voraciously on *A. tsugae* eggs (Montgomery and Keena 2011, Limbu et al. 2015). This predator's phenology is closely aligned with that of *A. tsugae*; *S. camptodromus* eggs diapause while *A. tsugae* are in summer aestivation, hatching in spring as *A. tsugae* begin laying eggs (Keena et al. 2012). Also, the lower temperature threshold for development of *S. camptodromus* larvae is 5°C, which closely matches that of *A. tsugae* progeny, making it an attractive choice for biological control in the northeastern United States (Limbu et al. 2015).

Scymnus camptodromus is found over a broad geographic region and diverse habitats in its native range, yet unlike some *Scymnus* (*Neopullus*) species collected from China, it was not found in association with host trees other than hemlock, suggesting it may be a specialist predator of *A. tsugae* (Montgomery and Keena 2011). However, as with introduction of any classical biological control agent, potential ecological impacts of the introduced species must be explored, particularly in respect to nontarget species (Bellows 2001, Van Lenteren et al. 2003). We studied the host range of *S. camptodromus*, including the possibility that it might feed on sympatric adelgid species, as well as four nonadelgid prey items, including *Paraprociophilus tessellatus* Fitch (Woolly alder aphid) in choice and no-choice tests. *Paraprociophilus tessellatus* is an important consideration for nontarget feeding, as the larvae of the only known carnivorous butterfly *Feniseca tarquinius* F. (Lepidoptera: Lycaenidae) in the continental United States feed almost exclusively on *P. tessellatus* (Scott 1986, Butin et al. 2004, Hall et al. 2007). These data were incorporated into an environmental assessment report for consideration for the release of *S. camptodromus* for control of *A. tsugae* in the field.

Materials and Methods

Predator and Prey Source

Scymnus camptodromus adults were collected in 2006–2007 from different geographic locations in China and transported to the USDA Forest Service quarantine facility in Ansonia, CT, under permit. Voucher specimens of adults were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT. Five geographic populations of *S. camptodromus* were used in

this study (Keena et al. 2012), including DGS (CHINA:Sichuan: 5-X-06, 5-11-XI-06, 26-IV-07), MNP (CHINA:Yunnan: 20-IV-07, 13-VI-07, 23-XI-07), NBG (CHINA:Sichuan: 5-X-06, 5-11-XI-06, 26-IV-07), LP (CHINA:Yunnan: 23-IX-05, 10-VI-07, 20-IX-07), and LJS (CHINA:Yunnan: 21-22-IV-07, 25-V-07, 11-VI-07, 21-IX-07). The strains used in any one experiment depended on which strain had adults available to test at the time. Equal numbers of predators from each province were evaluated in each test whenever possible.

Information on test prey with the rationale for testing a given prey species is shown in Table 1. The *A. tsugae* used in this study were collected from *T. canadensis* trees in 2007 and 2008 from the vicinity of Raleigh, NC, and in 2009 and 2010 from Rothrock State Forest, PA. *Scymnus camptodromus* was evaluated for nontarget impacts on three sympatric adelgids collected in April (overwintering adelgid generation) or June (second adelgid generation) of 2007, 2010, and 2015. *Pineus strobi* (Hartig) (Hemiptera: Adelgidae) (pine bark adelgid) were collected from *Pinus strobus* L. in Hamden, CT, Ansonia, CT, Perry Township, PA, and Yale Meyers Forest in CT. *Adelges cooleyi* (Gillette) (Cooley spruce gall adelgid) were collected from *Pseudotsuga menziesii* (Mirb.) Franco in Hamden, CT, and *Adelges laricis* Vallot (Hemiptera: Adelgidae) (larch adelgid) from the Lake Watrous area, CT, from *Larix kaempferi* (Lamb.) Carr. The predator was also tested using four nonadelgid species: *Fiorinia externa* Ferris (Hemiptera: Diaspididae) from *A. tsugae* in Mile Run exit, PA; *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae) (woolly apple aphid), from *Malus* species Borkh., in the Soergel Orchards area, PA; *P. tessellatus* (woolly alder aphid) from *Alnus serrulata* (Ait.) Willd in Scotia Barrens, PA, collected in February 2008; and *Aphis gossypii* Glover (Hemiptera: Aphididae) (cotton aphid) obtained from honeydew melon (*Cucumis melo* L.) plants from a lab colony maintained at Penn State University (University Park, PA) in March 2015.

Prey Preference (Choice Tests)

Choice tests were conducted in 150- by 15-mm Petri dishes with filter paper placed in the bottom. Host material with prey items was placed inside the Petri dish by trimming them to approximately equal size to minimize bias based on the amount of cover for prey or predator. All samples in each test were placed on top of the filter paper and arranged around the edges, making sure each sample touched the other samples as little as possible. The number of prey items presented to each predator was equalized as much as possible. Prior to placement in the choice test, each foliage sample was cleaned of any predators and other insects at the time it was collected.

The sex of each beetle, the foliage sample type, and the number of prey items present on each type of foliage were recorded prior to the start of each test. Each adult *S. camptodromus* was placed in the center of the test arena, equidistant from each sample, the lid of the Petri dish was closed, and the dish edges were sealed with parafilm or tape to prevent insects from escaping. To maintain humidity the Petri dishes were placed in a plastic box on a screen over water and held in an incubator at 15 ± 1°C with a photoperiod of 12:12 (L:D) h for 48 h (four-way choice tests) or 72 h (three-way choice tests).

After 48 or 72 h, the predators were removed, and the final location of each predator (on which sample or on the filter paper) was recorded. The predators were returned to holding cages, and the number of prey items remaining on each sample was recorded. Oviposition by *S. camptodromus* was noted and recorded.

Table 1. Test prey on associated host plant, the native range of test prey, and rationale for selection of prey item used in host specificity tests

Prey	Host plant	Native range	Rationale for testing
<i>Adelges tsugae</i> (hemlock woolly adelgid)	<i>Tsuga canadensis</i>	Japan	Target pest
<i>Pineus strobi</i> (pine bark adelgid)	<i>Pinus strobus</i>	Eastern North America	Taxonomic similarity
<i>Adelges cooleyi</i> (Cooley spruce gall adelgid)	<i>Pseudotsuga menziesii</i>	Western North America	Taxonomic similarity
<i>Adelges laricis</i> (larch adelgid)	<i>Larix kaempferi</i>	Europe	Taxonomic similarity
<i>Fiorinia externa</i> (elongate hemlock scale)	<i>T. canadensis</i>	Japan	Co-occurs with <i>A. tsugae</i> on same host
<i>Paraprociophilus tessellatus</i> (woolly alder aphid)	<i>Alnus serrulata</i>	Eastern North America	Ecological significance
<i>Eriosoma lanigerum</i> (woolly apple aphid)	<i>Malus domestica</i>	North America	Similar to woolly alder aphid
<i>Aphis gossypii</i> (cotton or melon aphid)	<i>Cucumis melo</i>	Worldwide	Another aphid species

Native range information for adelgids derived from (Havill and Footitt 2007).

Four-Way Choice Tests

To evaluate the feeding preference of adult *S. camptodromus* among sympatric adelgid species and for different adelgid stages, choice tests were conducted by giving each *S. camptodromus* adult a choice of four prey items (*A. laricis*, *P. strobi*, *A. tsugae*, *A. cooleyi*) simultaneously without removing the mature adelgids that continued laying eggs. As the choice test was performed with ovipositing adelgids, it was only held for 48 h to minimize oviposition and egg eclosion. Additionally, based on availability at the time of the test, the overwintering generation of *P. strobi*, *A. tsugae*, and *A. cooleyi* and the second generation of *A. laricis* were used. A 1-cm-long piece of infested hemlock, a 1-cm² piece of *P. strobi* excised infested bark, one infested needle of *P. menziesii*, and 3–5 infested needles of *L. kaempferi* were spaced evenly around the margins of the Petri dish. There were 10 replicates for this experiment, which included five MNP strain and five DGS strain females. After 48 h, the remaining adults, eggs, and crawlers were counted, and the remaining crawlers were subtracted from the eggs left in the test. Because ovipositing prey adults were present during the course of this experiment, we first sought to determine if there were differences in oviposition by prey species and prey generation, which could alter subsequent analyses. To estimate the average number of eggs laid by each adelgid in 48 h, all eggs were removed from 10 adelgid females of each species and held in the same containers and conditions as used in the choice test. In this analysis, the number of eggs laid by prey and prey generation was transformed using $\log_{10}(y + 1)$ and subjected to an analysis of variance.

Total eggs consumed of each adelgid species by each *S. camptodromus* adult was calculated by summing the number of eggs at the start plus the average number of eggs laid by a female of that species and then subtracting the number of eggs and nymphs in the container after 48 h. The number of nymphs (nymphs at the start plus hatched eggs minus nymphs at the end) and adults consumed was also recorded. The fate of an egg or nymph was considered to have a binary response: consumed or not consumed, and analyzed using logistic regression while accounting for the number of eggs presented to each *S. camptodromus* adult, given that these counts were not always equal among predators (although we made every effort to use approximately the same number of eggs). We chose this statistical approach over ordinary least squares (OLS) because the number of cases of consumed eggs were often very small for some prey species (i.e., <10), which is known to bias results obtained from OLS (Coxe et al. 2009). The significance of the main effect of prey was based on the likelihood ratio chi-squared (G^2), and odds ratios were computed after decomposing G^2 into nonsignificant components (Agresti 1990). Statistical analyses were conducted in R (R Development Core Team 2011).

Three-Way Choice Tests

Additional choice tests with *S. camptodromus* adults were conducted in comparison with *A. tsugae* by giving each predator a choice of three adelgid species at a time using *A. laricis*, *P. strobi*, and *A. cooleyi*. During the four-way tests, the predator did not show a preference for mature adelgids, so they were removed from the foliage samples to prevent further oviposition. However, as all four adelgid prey items were not available simultaneously, three-way tests were performed. To obtain sufficient sample sizes, both male and female adults of the MNP strain were used in these tests. The tests were run for 72 h (rather than the 48 h for the four-way tests) to provide more opportunity for the *S. camptodromus* females to lay eggs. Infested foliage samples were 3–15 cm in length (total length of all pieces of each foliage type in the dish was similar), with the ends of each wrapped in damp cotton with parafilm over it to preserve moisture and prevent the predators from drowning. The number of adelgid eggs present in each sample was kept as equal from sample to sample as possible, averaging 50–80 eggs per sample. The number of eggs of each prey item on their respective foliage at the start of each test was recorded, and all other adelgid life stages were removed. Each test arena contained *A. tsugae* and two other adelgid species. Tests were categorized as Set A, B, or C, depending on the composition of adelgids (Set A = *A. tsugae*, *A. laricis*, and *A. cooleyi*; Set B = *A. tsugae*, *P. strobi*, and *A. cooleyi*; and Set C = *A. tsugae*, *P. strobi*, and *A. laricis*). There were four replicates of Sets A and C and 11 replicates of Set B, based on availability of each adelgid species. Crawlers present at the start of the test were removed, and any crawlers present at the end of the test were subtracted from the number of eggs left after 72 h.

The statistical analyses of these data were conceptually the same as in the four-way choice tests with the exception of the inclusion of the main effect of prey set (A, B, or C), and the interaction of the two main effects (prey and prey set). The specific hypotheses tested in our logistic regression analysis were 1) predation does not vary among hosts, and 2) predation on hemlock woolly adelgid does not vary by the set composition of hosts. When appropriate, odds ratios were estimated, and post hoc tests were conducted by partitioning G^2 into nonsignificant components (Agresti 1990).

Paired Choice Tests

To further examine the prey preference of *S. camptodromus*, we also conducted paired choice tests when given a choice of *A. tsugae* and *F. externa*, or *A. tsugae* and *E. lanigerum*. *F. externa* and *A. tsugae* can coexist in hemlock trees, and an arboreal population of *E. lanigerum* and the progrediens generation of *A. tsugae* can co-occur. Thus, these prey species were tested in a paired choice test. A mixture of males

and females from the MNP strain of *S. camptodromus* was used for these tests. The experimental set-up was the same as described above for the three-way choice tests, but only *A. tsugae* and one other prey type was offered using 10 replicates of each combination. The eggs of *F. externa* and nymphs of *E. lanigerum* were counted at the beginning and end of the 72-h test. Data were analyzed using a paired Student's *t*-test using JMP 9 (SAS Institute 2012).

Prey Acceptance (No-Choice Tests)

No-choice feeding tests were also performed using adelgid prey items (*A. cooleyi*, *P. strobi*, *A. laricis*, and *A. tsugae*) and nonadelgid prey items (*A. gossypii*, *P. tessellatus*, *F. externa*, and *E. lanigerum*). We used 55- by 15-mm Petri dishes with filter paper placed in the bottom and sealed with parafilm, except for the test with *A. gossypii* where we used 50- by 9-mm Petri dish with a tight fitting lid and a layer of damp cotton in the bottom so the prey could not escape. Each of these tests was run for 48 h.

To test the adelgid species, *S. camptodromus* adults were presented with either 40 adelgid eggs alone spread out on the filter paper or 40 eggs with intact ovipositing adelgid(s) on their host material. The adelgid tests used the overwintering generation and second generation (as a separate treatment) of *A. laricis* and *A. tsugae*, the second generation of *A. cooleyi*, and the overwintering generation of *P. strobi*. Each adelgid species and generation was tested with and without host plant material. Adelgid-infested host material consisted of 1–2 cm of a *T. canadensis* branch with needles, a 0.5 cm length of *P. menziesii* with four needles, five to seven needles (second generation) or one to three whorls with needles (overwintering generation) of *L. kaempferi*, and either a 1-cm² piece of excised bark or a 7- by 3-cm bolt of *P. strobus* (the latter being contained in a 7 cm high-by-7 cm diameter plastic box with a single screen vent instead of the Petri dish). Five adults from Sichuan Province (DGS or NBS strain) and five from Yunnan Province (MNP or LJS strain) were used in each test with the second generation of adelgids. All but one or two of the adults used in tests of the overwintering generation were from Sichuan (LP), and the rest were from Yunnan (DGS or NBS strain).

The no-choice tests with the nonadelgid prey were only performed with host plant material present and with females from either the Sichuan (DGS) or Yunnan Province (MNP). Different strains of the predator were used at various times depending on availability at the time of the test. To accommodate the size differences between the predator and various nonadelgid prey items, we used different life stages (early instars, late instars, and adults) of *P. tessellatus* and *E. lanigerum* and adults only of *F. externa*. There were 10 replicates for each of these types of prey. The tests on *A. gossypii* were also performed with 2-cm-diameter disks of either melon or hibiscus host plant material placed on moist cotton. Twenty-five replicates were performed: Fifteen with melon and 10 with hibiscus plants, each containing 40–45 first-instar *A. gossypii* nymphs. The number of prey fed upon or consumed was recorded.

The effect of adelgid prey items on feeding preference of the predator was evaluated using the same method as used for the four-way choice tests. As tests were done with and without host material, the main effects of the host material and prey item, and their interaction were analyzed using logistic regression analysis in which significance was based on G^2 . Control for egg laying was done in the same manner as the four-way choice test because ovipositing adelgids were present.

Prey Suitability

We evaluated the ability of *S. camptodromus* to complete larval development by feeding only on *P. strobi* (needles and bark of

P. strobus) or *A. laricis* (on *L. kaempferi* foliage). The *P. strobi* and *A. laricis* were chosen because they were, with the exception to *A. tsugae*, the most and least preferred prey items in the four-way choice test, respectively. Newly hatched *S. camptodromus* larvae from the DGS or MNP strains (depending on availability) were immediately transferred individually to a rearing container containing prey items and observed until they died or reached the adult stage. Different rearing containers were used for the two prey types because of differences in needle size of the host plant. A Petri dish (150- by 15-mm sterile Petri dishes; www.daigger.com, EF1630C) was used for *P. strobi* and a clear 59-ml soufflé cup with a clear 2.5-cm-diameter lid (Solo, Eastern Bag and Paper, CT) for *A. laricis*. Each container consisted of filter paper placed at the bottom, and the adelgid-infested host material was placed on top of the filter paper. Foliage that contained sufficient adelgid eggs (based on choice and no-choice tests described above) was used and checked thoroughly for other predators before presenting to the *S. camptodromus* larvae. The larvae were reared in an environmental chamber at 20°C, with a photoperiod of 12:12 (L:D) h and an average humidity of $80 \pm 5\%$. The foliage was checked every other day for prey items and predator condition and changed when needed. Due to limited availability of *P. strobi* and *A. laricis* during peak larval hatch of *S. camptodromus*, the sample size was 10 beetle larvae on each of the prey items. Survival, the number of days to reach adulthood, and size of any eclosed predator adults, were recorded. Means and standard deviations were calculated for days to adult and adult sizes. Also, chi-square statistics were performed to determine if survival to adulthood on both prey items was different at $P \leq 0.05$ from the 67% survival that was reported on *A. tsugae* (Limbu et al. 2015).

Results

Choice Test With Four Adelgid Species

As ovipositing adelgid species were present during the 48-h test period, the effects of prey species and prey generation on adelgid oviposition were examined. We found that the mean oviposition by adelgids was 2.62 ± 0.42 eggs, which was not different by prey species ($F = 1.64$; $df = 3, 45$; $P = 0.19$) or generation ($F = 1.37$; $df = 1, 45$; $P = 0.25$).

Predation differed significantly among adelgid species (Fig. 1). *Scymnus camptodromus* adults were 4.0 times (95% CI: 2.7, 5.8) more likely to consume *A. tsugae* than *P. strobi*, and 251 (95% CI: 34.9, 999.9) times more likely to consume *A. tsugae* than the combined group of *A. cooleyi* and *A. laricis*. Similarly, the predator adults were 62.9 (95% CI: 8.6, 460.4) times more likely to consume *P. strobi* than the combined group of *A. cooleyi* and *A. laricis*. Consumption of *A. cooleyi* and *A. laricis* did not differ from each other ($G^2 = 1.8$, $P = 0.17$), which as a group differed from *P. strobi* ($G^2 = 34.5$; $P < 0.01$) and *A. tsugae* ($G^2 = 40.1$, $P < 0.01$). Among alternative adelgid hosts, *P. strobi* was most preferred, but the predator still strongly preferred *A. tsugae* over *P. strobi* ($G^2 = 5.2$, $P = 0.022$).

Choice Tests With Three Adelgids

There was a significant interaction of prey-by-set composition, indicating that predation of *A. tsugae* in each set depended on the combination of prey items present. Consumption of *A. tsugae* eggs was 1.9 (95% CI = 1.2, 2.8) times more likely to occur when *A. tsugae* was exposed to the predator in combination with *P. strobi* and *A. cooleyi* (Set B), or with *P. strobi* and *A. laricis* (Set C), than when combined with *A. laricis* and *A. cooleyi* (Set A). There was no

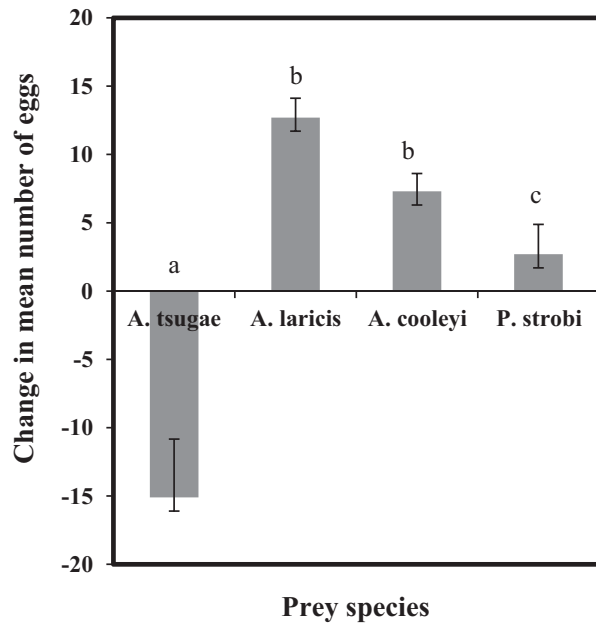


Fig. 1. Reduction in mean number of adelgid eggs (\pm SE) after 48 h when four adelgid species were provided simultaneously to *S. camptodromus*. Different letters represent significant differences in mean egg reduction ($G^2 = 44.5$; $P < 0.01$).

significant difference in *A. tsugae* egg consumption between sets B and C ($G^2 = 0.65$, $df = 1$, $P = 0.4178$), which both experienced significantly higher predation than Set A ($G^2 = 9.9$, $df = 1$, $P = 0.002$; Fig. 2).

Paired Choice Tests

When presented with the choice of *F. externa* and *A. tsugae*, the mean number of *A. tsugae* eggs consumed by adult *S. camptodromus* was 15.1 ± 2.9 (mean \pm SEM) over 72 h, which was 151 times greater than the mean number of *F. externa* consumed (0.1 ± 0.1). Also, the mean number of *A. tsugae* eggs consumed was 19 times higher (19.3 ± 2.9) than the mean number of *E. lanigerum* consumed (1.0 ± 0.44).

No Choice Test With Adelgid Prey Items

There was a significant effect of the presence of host plant material with adelgid prey ($G^2 = 26.9$, $P < 0.01$), and a significant effect of adelgid species ($G^2 = 7.8$, $P = 0.04$) on predation, but no significant host plant material by prey interaction ($G^2 = 4.7$, $P = 0.19$).

Holding host plant material as a main effect, *S. camptodromus* adults were 3.3 (95% CI: 2.9–3.7) times more likely to consume adelgid prey (including alternate prey) in the absence of host material than with host material present. Considering adelgid species as a main effect, *S. camptodromus* was equally likely to consume *A. cooleyi*, *P. strobi*, and *A. laricis* ($G^2 = 1.2$, $P = 0.28$), which as a group differed significantly from *A. tsugae* ($G^2 = 6.2$, $P = 0.013$). Predators were 1.9 (95% CI: 1.7, 2.1) times more likely to consume *A. tsugae* than the combined group of *A. laricis*, *A. cooleyi*, and *P. strobi*.

The effect of generation of *A. tsugae* was explored in two separate cases: with and without host material present. There was a significant generation effect with ($G^2 = 14.6$, $P < 0.01$) or without ($G^2 = 18.5$, $P < 0.01$) host material present; the predators were 7.6 (5.6, 10.2) times more likely to eat the overwintering generation than the second generation. With host material absent, there was

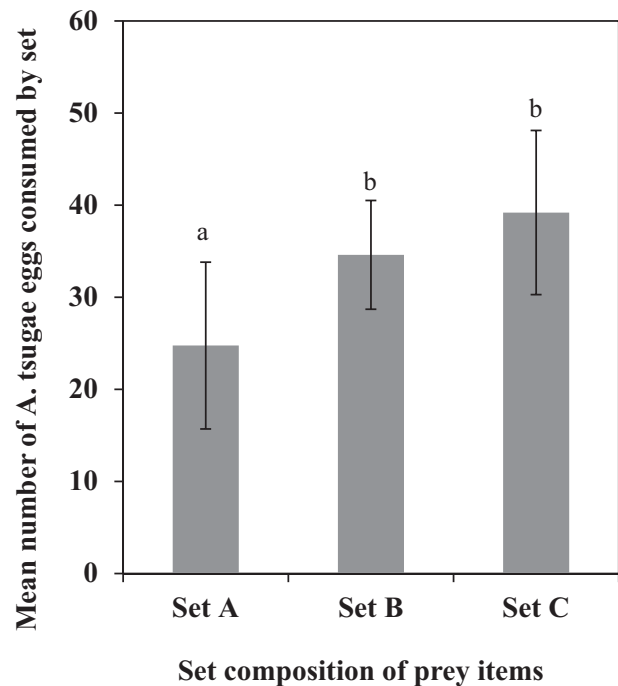


Fig. 2. Influence of prey combination on the mean number (\pm SE) of *A. tsugae* eggs consumed by *S. camptodromus*. Prey combination presented as Set A (*A. tsugae*, *A. laricis*, and *A. cooleyi*), Set B (*A. tsugae*, *P. strobi*, and *A. cooleyi*), and Set C (*A. tsugae*, *P. strobi*, and *A. laricis*). Different letters represent a significant difference in mean number of *A. tsugae* eggs consumed by *S. camptodromus* within each set ($G^2 = 14.1$, $df = 3$, $P = 0.003$).

also a significant generation effect ($G^2 = 18.5$, $P < 0.01$), with predators 25.9 (16.0, 41.9) times more likely to eat the overwintering generation than the second generation.

For *A. laricis*, there was no significant generation effect in the presence ($G^2 = 0.02$, $P = 0.88$) or absence ($G^2 = 1.05$, $P = 0.31$) of the host material.

No Choice Test With Nonadelgid Prey Items

When *P. tessellatus* or *E. lanigerum* were presented as the only prey species, adult *S. camptodromus* exhibited no evidence of feeding. However, when *F. externa* was presented as the only choice, one prey item was chewed upon in one of the tests but was not consumed entirely. On average, with host material present, 0.32 ± 0.031 nymphs of *A. gossypii* were consumed per predator adult over 48 h.

Prey Suitability

Of the 10 *S. camptodromus* neonate larvae reared exclusively on *P. strobi*, only two (less than expected, Person $\chi^2 = 44.94$, $df = 1$, $P < 0.0001$) pupated and eclosed to adult, which required an average of 28 ± 1.0 d. On larch, only one larva out of 10 survived (less than expected, Person $\chi^2 = 68.61$, $df = 1$, $P < 0.0001$) and required 44 d to reach adulthood. The size of the two adults reared on *P. strobi* was 2.48 ± 0.2 mm in length and 1.37 ± 0.1 mm in width, whereas the size of one adult reared on *A. laricis* was 2.19 mm in length and 1.07 mm in width.

Discussion

Nontarget testing indicated that *S. camptodromus* preferred *A. tsugae* over the alternative adelgid and nonadelgid prey items evaluated

in this study. Although, there was evidence that this predator will feed on other adelgid species to some extent, the predators consistently ate significantly more *A. tsugae* than any other adelgid species offered. We conclude, therefore, that release of *S. camptodromus* would not present a significant risk to nontarget species. This is in contrast to the predators *Scymnus ningshanensis* (Yu & Yao) from China and *S. tsugae* from Japan, which were released against *A. tsugae* in the eastern United States; these *Scymnus* species were shown to feed equally on *A. tsugae* and *P. strobi* or *A. cooleyi* eggs in paired choice tests performed in a laboratory (Butin et al. 2004).

In a prey suitability study to determine if the predator was able to complete larval development on an alternative prey item, we found that *S. camptodromus* larvae have a limited ability to complete development when fed only on *P. strobi* or *A. laricis*. The development time for the predator larvae to reach adulthood was 15 d longer on *A. laricis* at 20°C than larvae reared on *A. tsugae* at the same temperature (Limbu et al. 2015). Also, the adults reared on *P. strobi*, or *A. laricis* were smaller compared with the adults reared only on *A. tsugae* (Limbu et al. 2015). These findings suggest that the majority of *S. camptodromus* larvae would not reach adulthood in the field feeding exclusively on these alternative adelgid species, yet those that did reach the adult stage would likely experience fitness costs.

A small degree of feeding on *P. strobi* was observed, indicating the potential for *S. camptodromus* to survive during the summer months in the absence of *A. tsugae*. Using this prey source, *S. camptodromus* adults might be able to survive the summer until *A. tsugae* begins to lay eggs again in the fall. *Pineus strobi* is also considered a pest in most of its current range, so predation by *S. camptodromus* on this adelgid species may be of benefit by helping to manage *P. strobi* as well as *A. tsugae* (Raske and Hudson 1964). An additional consideration is that the likelihood of *S. camptodromus* larvae encountering prey on trees other than hemlock is very small, as this predator exclusively chose hemlock-infested foliage for oviposition in our trials. Also, during the no-choice tests on aphids, the predators were observed to avoid the host plant materials (hibiscus and melon) on which the aphids were feeding, suggesting that *S. camptodromus* is not likely to encounter nontarget pests such as aphids. These behaviors explain in part why this predator was never recovered from alternative tree species other than hemlocks in its native range (Montgomery and Keena 2011).

In similar studies on *Laricobius* species, both larvae and adults of *L. nigrinus* and *Laricobius osakensis* (Montgomery and Shiyake) consumed *P. strobi* eggs to some degree. In contrast to our results with *S. camptodromus*, *L. nigrinus* females were observed to oviposit on *P. strobi* in paired and no-choice tests, while *L. osakensis* oviposited only under no-choice conditions (Zilahi-Balogh et al. 2002, Vieira et al. 2011). However, unlike *S. camptodromus* both the *Laricobius* species larvae failed to develop to adult on this prey item alone (Zilahi-Balogh et al. 2002, Vieira et al. 2011), suggesting that in the event that *Laricobius* larvae eclosed on *P. strobi*, they would eventually die without reaching maturity unless they could find *A. tsugae*.

When presented with *F. externa*, *E. lanigerum*, or *P. tessellatus* as the only food source, *S. camptodromus* adults did not feed. This is in contrast to other predators of *A. tsugae* such as *S. ningshanensis*, *S. tsugae*, and *L. osakensis*, which were found to feed on *P. tessellatus* to some extent (Butin et al. 2004, Vieira et al. 2011). This is important to note due to the significance of *P. tessellatus* as the primary food source for *F. tarquinius*, which is the only carnivorous butterfly in the continental United States (Scott 1986, Butin et al. 2004). As expected, *S. camptodromus* is more specialized on

A. tsugae than the generalist lady beetle *Harmonia axyridis* (Pallas) that prefers *P. tessellatus* over *A. tsugae* (Butin et al. 2004).

Our findings indicate that *S. camptodromus* is a specialist on *A. tsugae*, particularly on the egg stage. Given the cold tolerance of *S. camptodromus* and its closely synchronized phenology with that of *A. tsugae* (Montgomery and Keena 2011), the absence of meaningful nontarget effects should make it an attractive candidate for biological control of *A. tsugae* in the northeastern United States.

Acknowledgments

We thank V. Sánchez and A. Vandel for assistance in the laboratory and Gary Thompson's lab for providing aphids for this study. Funding was provided in part by two USDA Forest Service Northeastern Area (NA) State and Private Forestry (S&PF) Technology Development competitive grants to K. H.: 13-CA-1142224-060—part of the Joint Hemlock Woolly Adelgid Initiative funding—and 09-DG-1142004-330 with allocation through PA DCNR to Improve Management of Hemlock Woolly Adelgid.

References Cited

- Agresti, A. 1990. Categorical data analysis. John Wiley and Sons, Inc., New York, NY.
- Bellows, T. S. 2001. Restoring population balance through natural enemy introductions. *Biol. Control* 21: 199–205.
- Butin, E., J. Elkinton, N. Havill, and M. Montgomery. 2003. Comparison of numerical response and predation effects of two coccinellid species on hemlock woolly adelgid (Homoptera: Adelgidae). *J. Econ. Entomol.* 96: 763–767.
- Butin, E., N. P. Havill, J. Elkinton, and M. Montgomery. 2004. Feeding preference of three lady beetle predators of hemlock woolly adelgid (Homoptera: Adelgidae). *J. Econ. Entomol.* 97: 1635–1641.
- Cheah, C.A.S.-J., and M. S. McClure. 1998. Life history and development of *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae), a new predator of the hemlock woolly adelgid (Homoptera: Adelgidae). *Environ. Entomol.* 27: 1531–1536.
- Cheah, C.A.S.-J., and M. S. McClure. 2000. Seasonal synchrony of life cycles between the exotic predator, *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae) and its prey, the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). *Agric. For. Entomol.* 2: 241–251.
- Cheah, C.A.S.-J., M. E. Montgomery, S. Salom, B. L. Parker, S. Costa, and M. Skinner. 2004. Biological control of hemlock woolly adelgid. For. Health Technol. Enterprise Team, U.S. Dep. Agric. Forest Service. FHTE-2004-04.
- Coxe, S., S. G. West, and L. S. Aiken. 2009. The analysis of count data: A gentle introduction to Poisson regression and its alternatives. *J. Pers. Assess.* 91: 121–136.
- Evans, A. M., and T. G. Gregoire. 2007. A geographically variable model of hemlock woolly adelgid spread. *Biol. Invasions* 9: 369–382.
- Flowers, R. W., S. M. Salom, and L. T. Kok. 2006. Competitive interactions among two specialist predators and a generalist predator of hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae) in south-western Virginia. *Agric. For. Entomol.* 8: 253–262.
- Ford, C. R., K. J. Elliott, B. D. Clinton, B. D. Kloeppel, and J. M. Vose. 2012. Forest dynamics following eastern hemlock mortality in the southern Appalachians. *Oikos* 121: 523–536.
- Hakeem, A., J. F. Grant, G. J. Wiggins, P. L. Lambdin, and J. R. Rhea. 2011. Establishment and coexistence of two predators, *Laricobius nigrinus* and *Sasajiscymnus tsugae*, introduced against hemlock woolly adelgid on eastern hemlock. *Biocontrol Sci. Tech.* 21: 687–691.
- Hakeem, A., J. F. Grant, G. J. Wiggins, P. L. Lambdin, F. A. Hale, D. S. Buckley, J. R. Rhea, J. P. Parkman, and G. Taylor. 2013. Factors affecting establishment and recovery of *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae), an introduced predator of hemlock woolly adelgid (Hemiptera: Adelgidae) on Eastern Hemlock (Pinales: Pinaceae). *Environ. Entomol.* 42: 1272–1280.

- Hall, D. W., M. Minno, and J. F. Butler. 2007. Harvester butterfly, *Feniseca tarquinius* (Fabricius) (Insecta: Lepidoptera: Lycaenidae: Melitinae), Vol. 2007. University of Florida IFAS Extension. EENY-404. (<http://edis.ifas.ufl.edu/pdf/FILES/in/in72700.pdf>) (accessed 19 November 2015).
- Havill, N. P., and R. G. Foottit. 2007. Biology and evolution of Adelgidae. *Annu. Rev. Entomol.* 52: 325–349.
- Havill, N. P., M. E. Montgomery, G. Yu, S. Shiyake, and A. Caccone. 2006. Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. *Ann. Entomol. Soc. Am.* 99: 195–203.
- Havill, N. P., L. C. Vieira, and S. M. Salom. 2014. Biology and control of hemlock woolly adelgid. FHTET-2014-05. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.
- Jones, C., N. P. Havill, J. Hanula, and S. Braman. 2014. Post release recovery of hemlock woolly adelgid predators in the North Georgia Mountains. *J. Entomol. Sci.* 49: 383–400.
- Keena, M. A., R. T. Trotter, C. Cheah, and M. E. Montgomery. 2012. Effects of temperature and photoperiod on the aestivo-hibernal egg diapause of *Scymnus camptodromus* (Coleoptera: Coccinellidae). *Environ. Entomol.* 41: 1662–1671.
- Limbu, S., M. Keena, D. Long, N. Ostiguy, and K. Hoover. 2015. *Scymnus camptodromus* (Coleoptera: Coccinellidae) larval development and predation of hemlock woolly adelgid. *Environ. Entomol.* 44: 81–89.
- Mausel, D. L., S. M. Salom, L. T. Kok, and G. A. Davis. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the eastern United States. *Environ. Entomol.* 39: 440–448.
- McClure, M. S. 1991. Density-dependent feedback and population-cycles in *Adelges tsugae* (Homoptera, Adelgidae) on *Tsuga canadensis*. *Environ. Entomol.* 20: 258–264.
- Montgomery, M. E., and M. A. Keena. 2011. Chapter 5: *Scymnus* (*Neopullus*) lady beetles from China, pp. 53–76. In R. Reardon and B. Onken (eds.), Implementation and status of biological control of the hemlock woolly adelgid. FHTET 2011-04. USDA Forest Service, Morgantown, WV.
- Montgomery, M. E., and S. M. Lyon. 1996. Natural enemies of adelgids in North America: their prospect for biological control of *Adelges tsugae* (Homoptera: Adelgidae), pp.89–102. In S. M. Salom, T. C. Tigner, and R. C. Reardon (eds.), Proceedings of the First Hemlock Woolly Adelgid Review, FHTET 96-10. USDA Forest Service, Charlottesville, VA.
- Montgomery, M. E., S. Shiyake, N. P. Havill, and R.A.B. Leschen. 2011. A new species of *Laricobius* (Coleoptera: Derodontidae) from Japan with phylogeny and a key for native and introduced congeners in North America. *Ann. Entomol. Soc. Am.* 104: 389–401.
- Onken, B., and R. C. Reardon. 2011. Implementation and status of biological control of the hemlock woolly adelgid. USDA/FHTET-2011-04). Forest Health Technology, Morgantown, WV.
- Parker, B. L., M. Skinner, S. Gouli, T. Ashikaga, and H. B. Teillon. 1999. Low lethal temperature for hemlock woolly adelgid (Homoptera: Adelgidae). *Environ. Entomol.* 28: 1085–1091.
- Preisser, E. L., K. L. Oten, and F. P. Hain. 2014. Hemlock woolly adelgid in the eastern United States: What have we learned? *Southeast. Nat.* 13: 1–15.
- Raske, A. G., and A. C. Hudson. 1964. The development of *Pineus strobi* (Hartig) (Adelgidae, Phylloxeridae) on white pine and black spruce. *Can. Entomol.* 96: 599–616.
- R Development Core Team. 2011. The R Project for Statistical Computing (www.r-project.org).
- SAS Institute Inc. 2012. JMP[®] 10. SAS Institute Inc, Cary, NC.
- Scott, J. A. 1986. *Feniseca tarquinius* harvester, pp. 356–357. The butterflies of North America. A natural history and field guide. Stanford University Press, Stanford, CA.
- Skinner, M., B. L. Parker, S. Gouli, and T. Ashikaga. 2003. Regional responses of hemlock woolly adelgid (Homoptera: Adelgidae) to low temperatures. *Environ. Entomol.* 32: 523–528.
- Story, H. M., L. C. Vieira, S. M. Salom, and L. T. Kok. 2012. Assessing performance and competition among three *Laricobius* (Coleoptera: Derodontidae) species, predators of hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae). *Environ. Entomol.* 41: 896–904.
- (USDA-FS) U.S. Department of Agriculture–Forest Service. 2012. Counties with established HWA populations 2012. (<http://na.fs.fed.us/fhp/hwa/maps/2012.pdf>) (accessed 22 January 2015).
- Van Lenteren, J. C., J. Bale, F. Bigler, H.M.T. Hokkanen, and A.J.M. Loomans. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. *Annu. Rev. Entomol.* 51: 609–634.
- Vieira, L. C., T. J. McAvoy, J. Chantos, A. B. Lamb, S. M. Salom, and L. T. Kok. 2011. Host range of *Laricobius osakensis* (Coleoptera: Derodontidae), a new biological control agent of hemlock woolly adelgid (Hemiptera: Adelgidae). *Environ. Entomol.* 40: 324–332.
- Wallace, M. S., and F. P. Hain. 2000. Field surveys and evaluation of native and established predators of the hemlock woolly adelgid (Homoptera: Adelgidae) in the southeastern United States. *Environ. Entomol.* 29: 638–644.
- Zilahi-Balogh, G.M.G., L. T. Kok, and S. M. Salom. 2002. Host specificity of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), a potential biological control agent of the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). *Biol. Control* 24: 192–198.