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Scymnus camptodromus (Coleoptera: Coccinellidae) Larval Development and Predation of Hemlock Woolly Adelgid (Hemiptera: Adelgidae)

SAMITA LIMBU,[1] MELODY A. KEENA,[2] DAVID LONG,[1] NANCY OSTIGUY,[1] AND KELLI HOOVER[1,3]

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ABSTRACT Development time and prey consumption of *Scymnus* (*Neopullus*) *camptodromus* Yu and Liu (Coleoptera: Coccinellidae) larvae by instar, strain, and temperature were evaluated. *S. camptodromus*, a specialist predator of hemlock woolly adelgid *Adelges tsugae* (Annand) (Hemiptera: Adelgidae), was brought to the United States from China as a potential biological control agent for *A. tsugae*. This beetle has been approved for removal from quarantine but has not yet been field released. We observed that temperature had significant effects on the predator's life history. The larvae tended to develop faster and consume more eggs of *A. tsugae* per day as rearing temperature increased. Mean egg consumption per day of *A. tsugae* was less at 15°C than at 20°C. However, as larvae took longer to develop at the lower temperature, the total number of eggs consumed per instar during larval development did not differ significantly between the two temperatures. The lower temperature threshold for predator larval development was estimated to be 5°C, which closely matches the developmental threshold of *A. tsugae* progrediens. Accumulated degree-days for 50% of the predator neonates to reach adulthood was estimated to be 424. Although temperature had a significant effect on larval development and predation, it did not impact survival, size, or sex ratio of the predator at 15 and 20°C. Furthermore, no remarkable distinctions were observed among different geographical populations of the predator.

KEY WORDS *Scymnus camptodromus*, *Adelges tsugae*, development time, prey consumption, degree-day

Introduction

The insect pest hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae), causes extensive decline of hemlock trees and threatens the sustainability of hemlock forests in the eastern United States. Native to Western North America and Asia, it was introduced in the eastern United States from Japan (Havill et al. 2006, Havill and Montgomery 2008) and is currently endemic to 19 eastern states (U.S. Department of Agriculture Forest Service [USFS] 2012, Preisser et al. 2014). Although *A. tsugae* is known to infest all hemlock species, it is more damaging to eastern hemlock, *Tsuga canadensis* (L.) Carriere, and Carolina hemlock, *Tsuga caroliniana* Engelmann, than other hemlock species. Hemlocks are important foundation tree species, dominating 1 million hectares of eastern U.S. forests (Domec et al. 2013). *A. tsugae* can multiply rapidly when hemlocks are healthy and producing new growth (McClure 1991). The insect injures trees by inserting their stylet bundle into plant tissue near where hemlock needle are attached and depleting nutrients from the xylem ray parenchyma cells (Young et al. 1995). Feeding damage results in yellowing and desiccation of hemlock needles, which can kill a tree in as little as 1–3 yr in its southern range and 5–15 yr in its northern range (Ellison et al. 2010).

Management approaches for *A. tsugae* include both chemical and biological control, but currently neither approach provides the level of population suppression needed (Onken and Reardon 2008, 2010). Biological control using a complex of natural enemies is thought to be a more sustainable, long-term solution for controlling this pest. There are no known parasitoids of *A. tsugae* and native natural enemies are generalists, which are unable to reduce pest populations to tolerable levels (Cheah et al. 2004). However, natural enemies of *A. tsugae* appear to contribute to natural control of this pest in its native range in Japan, China, and Western North America (Lu et al. 2002, Montgomery and Keena 2011, Vieira et al. 2013, Zilahi-Balogh et al. 2003). These include two species of *Laricobius* beetles and several species of coccinelids. Seven species of *Scymnus* (*Neopullus*) were found associated with hemlocks in China (Montgomery and Keena 2011,

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[1] Department of Entomology and Center for Chemical Ecology, The Pennsylvania State University, 501 ASI Bldg., University Park, PA 16802.

[2] U.S. Forest Service, Northern Research Station, 51 Mill Pond Rd., Hamden, CT 06514.

[3] Corresponding author, e-mail: kxh25@psu.edu.

Yu et al. 2000); three of these, *Scymnus (Neopullus) camptodromus* Yu and Liu (Sc), *Scymnus (N.) sinuanodulus* Yu & Yao (Ss) and *Scymnus (N.) ningshanensis* Yu & Yao (Sn), were collected from the Yunnan, Shaanxi, and Sichuan provinces of southwestern China and brought to the United States in 1995. *S. sinuanodulus* and *S. ningshanensis* were released in the United States in 2004 and 2007, respectively, but do not appear to have established (Montgomery and Keena 2011).

The third predator from this collection, *S. camptodromus*, differs from the other two *Scymnus* species in that its life cycle is strongly synchronized with that of *A. tsugae*. This predator has an unusual egg diapause that coincides with the summer aestivation of *A. tsugae*. Following egg diapause, *S. camptodromus* eggs hatch in the spring, coinciding with oviposition by their prey; the predator larvae begin feeding on *A. tsugae* eggs early in the season. In its native range this species is abundant over a broad geographic area and habitats, and thrives at variable prey densities. Unlike *S. sinuanodulus*, *S. camptodromus* was not found associated with any host plant other than hemlock in its native range (Montgomery and Keena 2011). As a first step to exploring the possibility of developing *S. camptodromus* as a biological control agent in the United States, nontarget testing was completed using choice, no choice, and predator development studies, showing that *S. camptodromus* prefers *A. tsugae*, although it will feed on other adelgids in no-choice situations, but not aphids (Montgomery and Keena 2011).

Larvae of *Scymnus (Neopullus)* species from China are voracious and, although observed to sometimes consume young crawlers or feed on other stages when they get larger, they prefer *A. tsugae* eggs (Montgomery and Keena 2011, Lu et al. 2002). Larval feeding behavior is distinct from that of adults. The beetle larvae feed by extra oral digestion (Delucchi 1954, Lu et al. 2002), while the adults chew on their prey, consuming the entire organism.

We evaluated *S. camptodromus* larval development and predation as a function of temperature among different beetle strains collected from two different regions in China. One of our goals was to contribute to a phenological model by establishing degree-day requirements for larval development. In addition, strain differences as a function of temperature in either developmental rate or number of prey consumed were evaluated to ensure close phenological and environmental matching for future field releases of this predator.

Materials and Methods

Source of Predators and Rearing. Different strains of *S. camptodromus* distinguished by geographic origin in China were collected and transported from China 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. Currently, these predators are being reared at the USDA Forest Service quarantine facility in Ansonia, CT, and at Pennsylvania State University, PA, after being released from quarantine. Three strains (geographic populations) of the predator, DGS (CHINA: Sichuan: 5-X-06, 5-11-XI-06, 26-IV-07), MNP (CHINA:Yunnan: 25-28-IX-05, 20-IV-07, 13-VI-07, 23-XI-07), and LJS (CHINA:Yunnan: 23-IX-05, 21-22-IV-07, 25-V-07, 11-VI-07, 21-IX-07) were used in this study (Keena et al. 2012).

This study began with eggs from the fifth generation of *S. camptodromus* reared in the laboratory. To simulate the conditions that initiate and then break diapause, each egg was held individually in a 0.5-ml clear microcentrifuge tube at 20°C for 29.5 d, 15°C for 52.5 d, 10°C for 50 d, 5°C for 116 d, and then transferred to 10°C for hatching (Keena et al. 2012). The predator eggs were observed daily at 10°C and newly hatched larvae were immediately transferred individually to a 59-ml soufflé cup with a clear 2.5 cm diameter lid (Solo, Eastern Bag and Paper, CT) for rearing. The opening of the cup was covered with a fine mesh cloth and was held in place by the lid with a 1 cm^2 "X" cut in the center to provide ventilation. A 2.4-cm-diameter piece of filter paper was placed in the bottom of the rearing containers.

Prey Provisioning. Hemlock twigs infested with *A. tsugae* were collected from two locations in central Pennsylvania: Bear Meadows (40.73 N, 77.75 W) and Penn State University's Russell E. Larson Agricultural Research Center (40.69 N, 77.96 W). Infested twigs were stored at 5°C until needed to delay egg hatch and prolong the period of *A. tsugae* oviposition. *A. tsugae* mothers were removed, and the number of eggs was counted on each twig before presenting it to the neonate beetles. As the predator larvae got older it became obvious which eggs had been fed upon; the eggs consumed by predator larvae were distinctly flat with all the egg contents sucked out, leaving an intact, yellowish orange chorion, whereas the hatched chorions were transparent and ruptured, so precounts were not necessary. Each twig in the larval rearing cup was checked every day to ensure sufficient eggs were still available and changed every three days, or after the predator molted to the next instar. The number of *A. tsugae* ovisacs and fresh eggs provided per day was increased with each successive instar.

Development Time of *S. camptodromus* Larvae by Temperature and Strain. A total of 150 *S. camptodromus* [DGS (51), LJS (74), MNP (25)] were randomly assigned to one of the two temperatures with a total of 81 larvae held at 15°C and 69 larvae at 20°C. The insects were maintained in environmental growth chambers at 32–40% relative humidity and a photoperiod of 12:12 (L:D) h; larval containers were misted with tap water twice daily. Each *S. camptodromus* larva was monitored daily for development and survival until they became adults. The end of each larval instar (1st to 4th), as well as the pupal stage, was determined based on first observation of an exuvium or an increase in the width of the head capsule. Developmental time in each instar or stage was then calculated as the time between these molts. After *S. camptodromus* adults

had sufficiently sclerotized, they were sexed by examining the shape of the last visible abdominal ventrite (Yu et al. 2000). Because the adults were too small to weigh, their size was quantified by measuring their length and width using digital calipers (Chicago Brand 50001, www.daigger.com, EF1630C). Fresh twigs with *A. tsugae* eggs were provided to the newly emerged adults. If the predator was found dead at any life stage, the date and cause of death, if it could be determined, were recorded.

Predation by Beetle Instar, Strain, and Temperature. To assess predation by *S. camptodromus*, numbers of *A. tsugae* eggs consumed by larvae from each strain and instar at 15 and 20°C were counted and recorded. Each time the *A. tsugae*-infested twigs were changed, the rearing container was examined for any dislodged *A. tsugae* eggs or hatched nymphs and then the twigs were observed under a dissecting microscope and all consumed *A. tsugae* eggs were counted and recorded. When the larva stopped feeding and transitioned to the pre-pupa, infested twigs were removed and replaced by fresh uninfested twigs to maintain humidity inside the cup and to provide a place for pupation. The nonfeeding pre-pupal stage wandered around searching for a suitable place to pupate, so it was difficult to distinguish the exact day the insect entered the pre-pupal stage; therefore, this stage was designated as part of the fourth instar.

Statistical Analysis. All statistical analyses were conducted using SAS (SAS Institute 2012). The influence of temperature and strain on survival of *S. camptodromus* from neonate to adult as well as sex ratio were determined using contingency analysis and Fisher Exact Tests. The number of *A. tsugae* eggs consumed per *S. camptodromus* instar, days spent in each instar, and eggs consumed per day within each instar were evaluated with the PROC UNIVARIATE procedure with the histogram option to assess the distributional fit of each response variable. Statistically, the Shapiro–-Wilk and the Anderson–Darling test were used to assess normality. However, in cases where no distributions met the normality assumption, histogram output was assessed visually to see which distribution most closely emulated the data.

Eggs consumed per instar, days spent in each instar, and eggs consumed per day within each instar were analyzed with a repeated measures completely randomized design.

$$Y_{ijk} = \mu + T_i + S_j + I_k + (TS)_{ij} + (TI)_{ik} + (SI)_{jk} + (TSI)_{ijk} + \varepsilon_{ijk} \quad \text{(Eqn 1)}$$

Where:
T_i = Temperature main effect
S_j = Strain main effect
I_k = Instar main effect
$(TS)_{ij}$ = Temperature and strain interaction
$(TI)_{ik}$ = Temperature and instar interaction
$(SI)_{jk}$ = Strain and instar interaction
$(TSI)_{ijk}$ = Temperature times strain times instar interaction

Number of eggs consumed per instar (note an egg was considered consumed even if it was not totally consumed by a first instar, as it would have rendered it unhatchable) and days spent in each instar were discrete variables, while eggs consumed per day within each instar was a continuous variable. The fixed effects in each model were temperature, strain, and instar. We used a generalized linear model via PROC GLIMMIX using a pseudo-likelihood estimation technique. To account for repeated measurements of organisms over instars, we used a repeated measures statement via the random residual option in PROC GLIMMIX. We assessed several covariance structures and selected the AR (1) (or ARH (1) when needed) because it provided the most random residuals, as fit statistics were not available. The response variables all had long right tails because of over-dispersion (the mean was several times smaller than the variance). Thus, we used a negative binomial distribution with a log link function for the discrete variables, eggs consumed per instar, and days spent in each instar. The lognormal distribution with a log link function was used for the eggs consumed per day, as it was the best fit for this continuous variable. The auto-regressive order 1 (AR (1)) covariance structure was used to account for the multiple measurements over instar for the number of days spent in an instar. However, after running the original AR(1) model for eggs consumed per instar and eggs consumed per day, there was a homogeneity of variance violation detected via the Levene's test, so the first-order autoregressive heterogeneous co-variance structure (ARH (1)) model was used instead for these two variables. Residuals were evaluated for normality and the homogeneity of variance assumption. The Kenward Rogers denominator degrees of freedom adjustment option was also used in the days in instar analysis only, and differences among means were determined by the LSMEANS option via the Tukey–Kramer Post hoc analysis. An alpha level of 0.05 was used in all analyses to assess significance.

The fixed effects of temperature, sex, strain, and their interactions on length and width of predators when they reached the adult stage were analyzed using PROC MIXED and the REML. Mean differences were determined following each analysis using the least squared means test with $\alpha = 0.05$ and the Bonferroni correction.

Estimating Degree-Day Requirements (DD) for Larvae. Development time to adulthood at 10, 15, 20, and 25°C was used to estimate the relationship between temperature and developmental rate (1/day). In a separate experiment, larval development of *S. camptodromus* fed *A. tsugae* prey from neonate to adult (time in instar was not documented) was evaluated at 10 and 25°C at the USDA Forest Service Northern Research Station in Ansonia, CT, using the same beetle strains and the same methods described above. These data were combined with the data from the developmental time experiment conducted at 15

and 20°C for degree-day (DD) estimates. The relationship between developmental rate (Y_{DR}) and temperature (T) was estimated using linear regression and is represented by the equation, $Y_{DR} = \beta_0 + \beta_1 xT$.

Development time to adulthood at 15, 20, and 25°C was used to calculate the degree-day requirement for *S. camptodromus*. The degree-day calculation did not include 10°C because responses to temperatures near a threshold often are more heterogeneous (Keena 2006). From the relationship between temperature and development rate, the lower temperature threshold (T_L) for development was calculated by setting the development rate to zero. Using the calculated T_L the number of degree-days required for each individual to reach adulthood was calculated. The relationship used to calculate degree day was as follows:

$DD = [\text{constant holding temperature (15, 20, 25°C)} - T_L] \times Dt$, where Dt is the total development time by each individual predator to reach adulthood at a constant temperature. The cumulative proportions of predators reaching adulthood after accumulation of a specific number of degree days were used to determine the relationship between DD and the proportion that became adults using non-linear regression and the Gompertz function, $P = \exp[-\exp(-bDD + a)]$ (Brown and Mayer 1988) with the Marquardt convergence method. Accumulated degree-days required for 10, 50, 90 and 99% of the predator population to reach adulthood were calculated.

Results

Effect of Temperature on Predator Survival and Development Time. Out of the 150 *S. camptodromus* larvae reared, 101 survived to adult. We did not observe a significant difference in larval survival (Fisher Exact Test; $P = 1.0$) and sex ratio (Fisher Exact Test; $P = 0.53$) between 15 and 20°C, nor was there an effect of strain on survivorship (Fisher Exact Test; $P = 0.60$). However, sex ratio differed by strain (Fisher Exact Test; $P = 0.002$); the ratio of females to males for DGS, LJS, and MNP were 1:1, 5:1, and 1:1, respectively.

The mean number of days spent in each life stage did not differ by strain as an overall effect, and there was no temperature × strain interaction (Table 1). However, as main effects temperature and instar had significant impacts, development time was slower at 15°C than at 20°C, and time spent in each life stage significantly increased with each subsequent life stage (Tables 1 and 2). For example, the predator spent significantly more time in the fourth instar compared with the other instars regardless of strain. The three-way interaction term was not significant, but there were significant temperature × instar and strain × instar interactions (Table 1). For example, *S. camptodromus* larvae spent significantly more time in all life stages at 15°C than 20°C, except the second instar, which did not differ at the two temperatures (Table 2). The strain × instar interaction indicated that no single strain consistently spent the longest or shortest amount of time in each life stage (Table 2).

Table 1. GLIMMIX model for mean development time of *S. camptodromus* after eclosion

Effect	*F*	Df	*P* > *F*
Temp	424	1	<0.0001
Strain	1.7	2	0.1835
Instar	1361	4	<0.0001
Temp × Instar	19	4	<0.0001
Temp × Strain	1.62	2	0.2014
Strain × Instar	2.65	8	0.0077
Temp × Strain × Instar	0.92	8	0.5009

See methods for details of analysis.

The total mean development time to adulthood was calculated at each temperature and for each strain. DGS, LJS, and MNP took 43.2 ± 0.83, 40.5 ± 0.39, 42.5 ± 0.54 d, respectively, to reach adulthood at 15°C and 29.5 ± 0.37, 28.8 ± 0.19, 29.7 ± 0.54 days, respectively, at 20°C. The average time to adult among all strains was 12 d longer at 15°C than at 20°C.

Predation by *S. camptodromus* Larvae. The mean number of *A. tsugae* eggs consumed by *S. camptodromus* by instar did not differ among strains or between temperatures as an overall effect and also there was no significant temperature × strain interaction (Table 3). As a main effect, egg consumption significantly increased with each subsequent instar from 31.9 ± 1.3 eggs in the first instar to 171 ± 7.0 eggs in the fourth instar, with no significant difference between the third and fourth instars (Table 4).

There was no significant three-way interaction effect on mean egg consumption by instar, but the temperature × instar and strain × instar interactions were significant (Table 3). For example, the mean number of eggs consumed within the first three instars did not differ between rearing temperatures; however, more eggs were consumed during the fourth instar at 15°C than at 20°C by strains LJS and MNP. Although there was a significant strain × instar interaction, the only discernable differences in mean egg consumption among strains across instars were that different strains showed slightly different trends in how much egg consumption increased through the predators' life cycle (Table 3). Most differences were, however, not statistically significant.

The mean number of *A. tsugae* eggs consumed from neonate to pupation by strain (DGS, LJS, and MNP) and temperature were 458 ± 17.9, 533 ± 25.6, and 535 ± 34.6, respectively, at 15°C, and 449 ± 22.5, 440 ± 14.8, and 494 ± 26.7, respectively, at 20°C. Because, the predators spent less time as larvae at 20°C (see above), we compared total predation per larva per day as a function of temperature, instar, strain, and their interactions (Table 5 and Fig. 1). We found that predation per day was also not different among strains and there was no three-way interaction. As a main effect temperature had a significant impact, the mean number of *A. tsugae* eggs consumed per day was significantly less at 15°C (22.9 ± 0.8 eggs) than at 20°C (31.4 ± 0.9 eggs; Fig. 1). Although larvae ate less per day and took longer to develop at the cooler temperature, the mean number of eggs consumed per

Table 2. Mean development time [mean days ± SE (*n*)] by life stage of *S. camptodromus* reared at 15 or 20°C after eclosion

Temp/strain	Instar				Pupal stage
	I	II	III	IV	
15°C					
DGS	6.1 ± 1.03e (21)	3.4 ± 1.04ghij(19)	4.2 ± 1.04f (18)	15.6 ± 1.05a (18)	14.0 ± 1.05ab (18)
LJS	5.3 ± 1.03e (28)	3.6 ± 1.03fgh(28)	3.9 ± 1.03fg (28)	13.9 ± 1.03ab (28)	13.7 ± 1.04ab (28)
MNP	5.3 ± 1.04e (12)	3.8 ± 1.04fgh(12)	3.8 ± 1.04fgh (12)	15.1 ± 1.05a (12)	14.6 ± 1.05a (12)
20°C					
DGS	3.6 ± 1.04fghi (17)	2.8 ± 1.04j(16)	3.1 ± 1.04hij (15)	8.9 ± 1.04cd (15)	11.1 ± 1.04bc (15)
LJS	3.6 ± 1.03fgh (26)	3.2 ± 1.03ghij (25)	3.0 ± 1.03ij (24)	8.8 ± 1.03d (23)	10.4 ± 1.04cd (23)
MNP	3.8 ± 1.05fgh (11)	3.2 ± 1.05ghij (10)	3.1 ± 1.05hij (10)	9.0 ± 1.05cd (10)	10.7 ± 1.06cd (9)

Sample size (*n*) is based on number of survivors. Because there were significant temperature × instar and strain × instar interactions (Table 1), all means were compared among each other. Means followed by a different letter within the table are significantly different from each other at $P < 0.05$ using Tukey–Kramer post hoc test.

Table 3. GLIMMIX model for mean number of hemlock woolly adelgid eggs consumed by *S. camptodromus* after eclosion

Effect	*F* value	df	*P* > *F*
Temp	5.07	1	0.1380
Strain	2.22	2	0.5310
Instar	392	3	< 0.0001
Temp × Instar	19.4	3	< 0.0001
Temp × Strain	1.14	2	0.2020
Strain × Instar	4.95	6	< 0.0001
Temp × Strain × Instar	1.46	6	0.0956

instar generally did not differ between these two temperatures (no significant temperature main effect). There were also significant instar × temperature and strain × instar interactions on mean egg consumption per day by instar (Table 5). For example egg predation per day was not different within the first, second, or fourth instars, but DGS consumed significantly fewer eggs per day in the third instar than other strains during the same life stage regardless of temperature.

Estimation of Degree-Day Requirement. Development time (in days) was inversely related to temperature; development rate (1/days) increased as the temperature increased ($F = 1526$; df = 1, 154; $P < 0.0001$; Fig. 2). The relationship between temperature and development rate to adulthood can be described as, $Y_{DR} = (0.0022 \pm 0.0001)T - 0.0116 \pm 0.0009$, where Y_{DR} is development rate and T is the corresponding temperature in degrees Celcius. The rate of development for *S. camptodromus* pupae was faster (steeper slope of the regression line) than the rate of development for larvae (Fig. 3). The lower development temperature threshold (T_L) for development to adult was calculated to be 5.18°C. The degree-day requirement for development from neonate to adult for 10, 50, 90 and 99 % of the *S. camptodromus* population was predicted as 397 ± 1.0, 424 ± 0.2, 467 ± 2.4, and 520 ± 5.0 DD, respectively (Fig. 4; $R^2_{adj} = 0.99$, $F = 3308$; df = 2, 18; $P < 0.0001$).

Effects of Temperature, Sex, and Strain on Adult Beetle Size. The length and width of *S. camptodromus* adults were unaffected by temperature (length: $F = 0.1$; df = 1, 99; $P = 0.96$; width: $F = 0.90$; df = 1, 99; $P = 0.34$), sex (length: $F = 0.88$; df = 1, 95; $P = 0.35$; width: $F = 2.46$; df = 1, 95; $P = 0.12$), or their interactions (Table 6). However, there was a significant difference by strain on the length ($F = 15.6$; df = 2, 95; $P < 0.001$) and width ($F = 19.5$, df = 2, 95; $P < 0.0002$) of the predators, with LJS being significantly larger (longer and wider) than DGS or MNP. To determine if this difference could be due to females in the population, which tend to be larger than males, we compared length and width of only females among strains, and obtained the same result (length: $F = 7.75$; df = 2, 63; $P = 0.001$; width: $F = 14.3$; df = 2, 63; $P < 0.0001$). There was also a significant relationship between length and width of the individual predators ($R^2_{adj} = 0.26$, $F = 35.3$; df = 1, 99; $P < 0.0001$).

Discussion

As expected, development rate (1/days) of *S. camptodromus* increased with increasing temperature and followed a linear relationship (Fig. 2). While temperature did not affect size or sex ratio of the predators at the temperatures tested, it did have significant effects on development and predation, but the impacts varied depending upon predator strain and instar. For example, on average strains LJS and MNP consumed more *A. tsugae* eggs in the fourth instar at 15°C than at 20°C, while this did not occur for DGS. It is clear, however, that there was no consistent effect of strain on development or predation. LJS were bigger as adults than the other strains regardless of temperature, yet the larvae of this strain ate the same number of eggs during development as did the MNP strain. Studies are underway to determine if there are any differences among strains in fecundity. Given that *S. camptodromus* adults reared in the lab were bigger than the same species reared under natural conditions (Yu et al. 1996), this may not hold in the field. In our study we also found that *S. camptodromus* adults were larger on average than was reported for *S. sinuanodulus* and *S. ningshanensis* (Montgomery and Keena 2011).

The lower temperature threshold for *S. camptodromus* development was close to 5°C, which is comparable with the lower threshold limit of 3.9°C for development of *A. tsugae* progrediens (Salom et al. 2002). At 25°C, however, very few *S. camptodromus*

Table 4. Mean number [mean ± SE (*n*)] of hemlock woolly adelgid eggs consumed by *S. camptodromus* by instar reared at 15°C or 20°C after larval eclosion

Temp/strain	I	II	III	IV
15°C				
DGS	36.0 ± 3.2g (23)	67.8 ± 6.0f (19)	144 ± 12.6abcd (18)	208 ± 18.0ab (18)
LJS	32.1 ± 2.4g (30)	81.2 ± 5.7ef (29)	178 ± 12.4ab (28)	220 ± 15.2ab (28)
MNP	31.2 ± 3.7g (12)	81.6 ± 8.9ef (12)	187 ± 19.8ab (12)	236 ± 24.9a (12)
20°C				
DGS	36.1 ± 3.6g (19)	81.4 ± 7.7ef (16)	164 ± 15.6abc (15)	168 ± 16.0abc (15)
LJS	29.2 ± 2.4g (28)	104 ± 8.0cdef (25)	211 ± 16.1ab (24)	96.4 ± 7.7def (23)
MNP	28.4 ± 3.6g (11)	102 ± 12.1cdef (10)	223 ± 25.7ab (10)	127 ± 15.0bcde (10)

Sample size (*n*) is based on number of survivors within each column. GLIMMIX was used to determine the effects of instar, strain, temperature, and their interactions on mean eggs consumption by the predator larvae. Because there were significant temperature × instar and strain × instar interactions on mean egg consumption (Table 3), all means were compared among each other. Means followed by a different letter within the table are significantly different from each other at $P < 0.05$ using Tukey–Kramer post hoc test.

Table 5. GLIMMIX model for mean number of hemlock woolly adelgid egg consumed per day by *S. camptodromus* after eclosion

Effect	*F* value	df	$P > F$
Temp	46.5	1	< 0.0001
Strain	1.31	2	0.2729
Instar	594	3	< 0.0001
Temp × Instar	7.1	3	0.0002
Temp × Strain	3.49	2	0.0329
Strain × Instar	5.66	6	< 0.0001
Temp × Strain × Instar	2.04	6	0.0609

larvae survived to adulthood, suggesting that the upper temperature threshold for larval development is near 25°C, which is comparable with the upper temperature threshold of 22–27°C for development of *A. tsugae* progrediens. Thus, the temperature thresholds for *S. camptodromus* are closely matched with those of its prey, suggesting that the predator may be able to survive where *A. tsugae* can thrive. At 20°C, development time of predator larvae from hatch to adult was comparable with that of the other two predators from this genus collected in China, i.e., *S. sinuanodulus* and *S. ningshanensis.* However, under the conditions of our study at 15°C, *S. camptodromus* developed faster compared with *S. sinuanodulus* (Lu and Montgomery 2001). *S. camptodromus* also developed faster than was reported for *Laricobius nigrinus* Fender (Coleoptera: Derodontidae). *L. nigrinus* was introduced as a biological control agent for *A. tsugae* and appears to have established in some parts of the eastern United States (Zilahi-Balogh et al. 2003).

Unlike other predators from China, *S. camptodromus* has a true aestival diapause, which is synchronous with the summer diapause of the *A. tsugae* neosistentes (Keena et al. 2012). *S. camptodromus* eggs do not hatch until *A. tsugae* oviposition begins in the late winter or early spring, ensuring that the developing larvae have sufficient food to reach adulthood. This strategy may explain, at least in part, why *S. camptodromus* was found in greater abundance in broader geographic regions and at variable *A. tsugae* densities in its native range compared with other native predators (Montgomery and Keena 2011).

Although *S. camptodromus* consumed greater numbers of *A. tsugae* eggs per day at 20 than at 15°C,

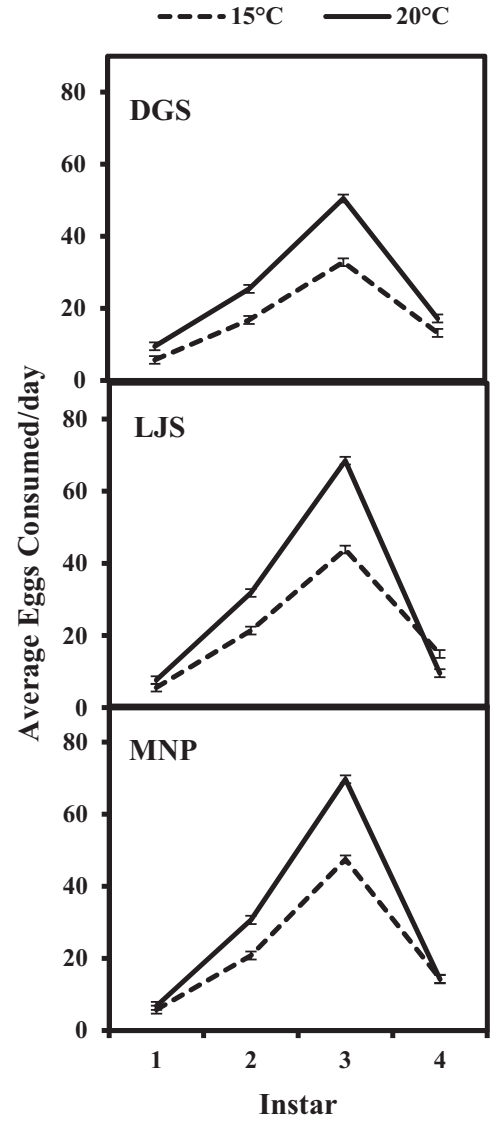

Fig. 1. Average eggs consumed per day among different *S. camptodromus* strains and instars. The solid line represents egg consumption per day at 20°C and the dashed line represents egg consumption per day at 15°C.

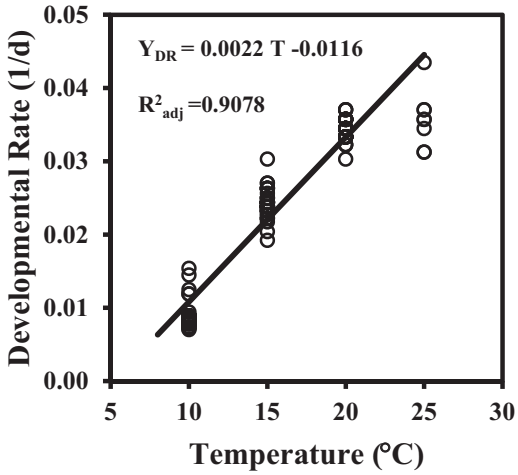

Fig. 2. Relationship between temperature and development rate (1/d) of *S. camptodromus* from neonate to adult. The line represents the simple linear regression combining all predator strains; some circles represent multiple data points at a given temperature due to overlap.

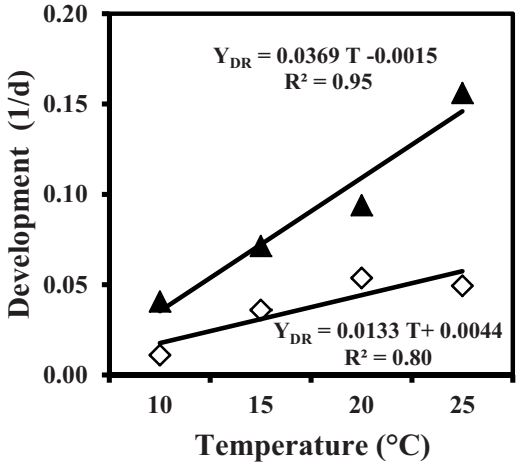

Fig. 3. Relationships between temperature and *S. camptodromus* larval and pupal rates (1/days) of development. Lines represent the estimated linear relationships combining all the predator strains (DGS, LJS, and MNP). Each diamond represents mean days to pupation and each triangle represents days as pupa at each temperature.

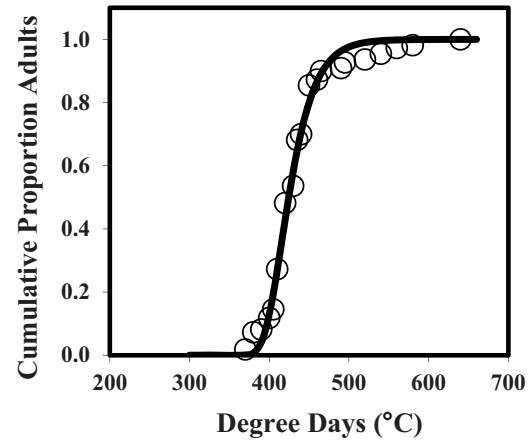

Fig. 4. Cumulative proportion of *S. camptodromus* neonates to reach adulthood over ccumulated degree-days. Open circles represent individuals reared at 15, 20, and 25°C; there is considerable overlap of circles.

Table 6. Mean size [mean ± SE (n)] of *S. camptodromus* following adult eclosion reared at 15°C or 20°C

Temp/strain	Avg length (mm)	Avg width (mm)
15°C		
DGS	2.68 ± 0.06a (15)	1.34 ± 0.02a (15)
LJS	2.83 ± 0.03b (27)	1.44 ± 0.02b (27)
MNP	2.58 ± 0.07a (12)	1.39 ± 0.02a (12)
20°C		
DGS	2.59 ± 0.07a (15)	1.36 ± 0.01a (15)
LJS	2.84 ± 0.02b (23)	1.45 ± 0.01b (23)
MNP	2.70 ± 0.05a (9)	1.41 ± 0.01a (9)

Sample size (*n*) is based on number of survivors to adult. PROC MIXED was used to determine the fixed effects of temperature, sex, strain, and their interactions on length and width of predators. Length and width of predator was not affected by temperature.Neither sex nor interaction between sex and strain affected predator size. In contrast, strain differences in size were apparent. Within a column, means followed by a different letter were significantly different at $P < 0.05$ with Bonferroni correction method.

overall egg consumption per instar was not different between the two temperatures. This can be explained by the fact that although larvae ate more *A. tsugae* eggs per day at 20°C, they took less time to develop at this temperature, thus ultimately requiring fewer eggs to complete development at the warmer temperature. Contrary to what we expected fewer hemlock woolly adelgid eggs were consumed in the fourth than the third instar (Fig. 1); however, this may have been an artifact of the experimental design. Because it was difficult to determine the specific day fourth instars entered the non-feeding pre-pupal stage, all larvae in the fourth instar were combined for analysis without separating the pre-pupal stage. On average predator larvae consumed 31.4 ± 0.91 *A. tsugae* eggs per day at 20°C, which is comparable with predation by the adults of this species reported previously (mean of 31 *A. tsugae* eggs per day) at 19°C (Zhao et al. 1998). The total average consumption by *S. camptodromus* larvae to complete development to adult at 15 and 20°C was 512 ± 16.0 and 454 ± 11.5 eggs, respectively, which is more than the reported 226 ± 18 and 252 ± 18 eggs consumed by *L. nigrinus* larvae at 12 and 18°C, respectively (Zilahi-Balogh et al. 2003), indicating that *S. camptodromus* has considerable potential for biological control of *A. tsugae*.

Field studies are needed to determine the upper temperature threshold for development of this predator, as laboratory conditions at a constant 25°C may have underestimated the upper threshold for *S. camptodromus* development, survival, or both. It is expected that *S. camptodromus* will benefit from its ability to develop at very low temperatures if released in the northeastern part of the *A. tsugae* range. These predators are also known to survive for more than one year in a laboratory setting (M. K., unpublished data). Further studies under field conditions are needed to determine the lower and higher temperature threshold for the adults in the eastern United States, which has implications for adult survival and reproduction over multiple years. Field studies to determine when *S. camptodromus* eggs will hatch under natural conditions (fluctuating temperatures and weather extremes) are also planned.

These findings on developmental rates, degree-day requirements, and predator consumption will inform confined release studies on predator–prey interactions and provide baseline data for developing mass rearing procedures and planning field releases. Moreover, these results provide needed information to help improve laboratory rearing. This study, although done under constant temperatures and humidity in a laboratory setting, shows that *S. camptodromus* can be an effective predator of *A. tsugae*; further studies are needed to verify these results under field conditions.

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References Cited

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. *In* R. Reardon and B. Onken (Tech. Coordinators), USDA Forest Service, Morgantown, WV, FHTET-2004-04.

Delucchi, V. (1954). *Pullus impexus* (Muls.)(Coleoptera, Coccinellidae), a predator of *Adelges piceae* (Ratz.) (Hemiptera, Adelgidae), with notes on its parasites. Bull. Entomol. Res. 45: 243–278.

Domec, J. C., L. N. Rivera, J. S. King, I. Peszlen, F. Hain, B. Smith, and J. Frampton. (2013). Hemlock woolly adelgid (*Adelges tsugae*) infestation affects water and carbon relations of eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*). New Phytol. 199: 452–463.

Ellison, A. M., A. A. Barker-Plotkin, D. R. Foster, and D. A. Orwig. (2010). Experimentally testing the role of foundation species in forests: the Harvard Forest Hemlock Removal Experiment. Methods Ecol. Evol. 1: 168–179.

Havill, N. P., and M. E. Montgomery. (2008). The role of arboreta in studying the evolution of host resistance to the hemlock woolly adelgid. Arnoldia 65: 1–9.

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.

Keena, M. A. (2006). Effects of temperature on *Anoplophora glabripennis* (Coleoptera: Cerambycidae) adult survival, reproduction, and egg hatch. Environ. Entomol. 35: 912–921.

Keena, M. A., R. T. Trotter III, 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.

Lu, W., and M. E. Montgomery. (2001). Oviposition, development, and feeding of *Scymnus (Neopullus) sinuanodulus* (Coleoptera: Coccinellidae): a predator of *Adelges tsugae* (Homoptera: Adelgidae). Ann. Entomol. Soc. Am. 94: 64–70.

Lu, W., P. Souphanya, and M. E. Montgomery. (2002). Descriptions of immature stages of *Scymnus (Neopullus) sinuanodulus* Yu and Yao (Coleoptera: Coccinellidae) with notes on life history. Coleop. Bull. 56: 127–141.

McClure, M. (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.), Proceedings, Symposium: Implementation and Status of Biological Control of the Hemlock Woolly Adelgid. FHTET 2011-04. USDA Forest Service, Morgantown, WV.

Onken, B. and R. Reardon. (2008). Fourth symposium on hemlock woolly adelgid in the Eastern United States, p. 299. FHTET-08-01. U.S. Department of Agriculture, Forest Service, Morgantown, WV.

Onken, B. and R. Reardon. (2010). Fifth symposium on hemlock woolly adelgid in the Eastern United States, p. 222. FHTET-10-07. U.S. Department of Agriculture, Forest Service, Morgantown, WV.

Preisser, E.L., K.L. Oten, and F. P. Hain. (2014). Hemlock woolly adelgid in the Eastern United States: What have we learned? Southwest. Nat. 13: 1–15.

Salom, S. M., A. S. Sharov, W. T. Mays, and D. R. Gray. (2002). Influence of temperature on development of hemlock woolly adelgid (Homoptera: Adelgidae) progrediens. J. Entomol. Sci. 37: 166–176.

SAS Institute. (2012). SAS® 9.3 In-Database Products: User's Guide, version 4th ed. SAS Institute, Cary, NC.

(USFS) US Department of Agriculture Forest Service. (2012). Forest Health Protection: Hemlock Woolly Adelgid Distribution Map. Forest Health Protection Service, Newtown Square, PA.

Vieira, L., A. Lamb, S. Shiyake, S. Salom, and L. Kok. (2013). Seasonal abundance and synchrony between *Laricobius osakensis* (Coleoptera: Derodontidae) and its prey, *Adelges tsugae* (Hemiptera: Adelgidae) in Japan. Ann. Entomol. Soc. Am. 106: 249–257.

Young, R. F., K. S. Shields, and G. P. Berlyn. (1995). Hemlock woolly adelgid (Homoptera: Adelgidae): Stylet bundle insertion and feeding sites. Ann. Entomol. Soc. Am. 88: 827–835.

Yu, G., D., Yao, and H., Liu (1996). The coccinellidae collected from Tsuga with *Adelges tsuga* Annand (Homoptera: Adelgidae). Sci. Silvae Sin 33: 432–440.

Yu, G., M. E. Montgomery, and D. Yao. (2000). Lady beetles (Coleoptera: Coccinellidae) from Chinese hemlocks infested with the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). Coleopt. Bull. 54: 154–199.

Zilahi-Balogh, G. M., S. M. Salom, and L. T. Kok. (2003). Development and reproductive biology of *Laricobius nigrinus*, a potential biological control agent of *Adelges tsugae*. Biocontrol 48: 293–306.

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