Effects of Temperature and Photoperiod on the Aestivo-Hibernal Egg Diapause of *Scymnus camptodromus* (Coleoptera: Coccinellidae)

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Environ. Entomol. 41(6): 1662–1671 (2012); DOI: http://dx.doi.org/10.1603/EN12217

ABSTRACT Three sequential studies were conducted on the interacting effects of exposure to low (5°C) temperature for 0, 7, 28, 56, or 84 d followed by incubation at 10, 15, or 20°C on the egg diapause of *Scymnus* (*Neopullus*) *camptodromus* Yu and Liu (Coleoptera: Coccinellidae). This beetle was imported from China as a potential biological control agent for hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae). Very few eggs laid and held at a constant 15 or 20°C showed any indication of development. Only eggs exposed to temperature combinations of 5 and 10°C had >50% hatch. Highest percent hatch and fastest development occurred when eggs were held at 5°C for 56 or 84 d followed by holding at 10°C. A model estimated the lower threshold for postdiapause development to be 2°C. The effect of temperature on egg hatch was similar at photoperiods of 12:12 and 16:8 (L:D) h, suggesting egg development is not governed by photoperiod or light exposure. Collectively these data indicate that *S. camptodromus* eggs laid in the spring and summer go through an aestivo-hibernal diapause that is maintained by warm temperatures and that development resumes when temperatures drop, in parallel with the development of hemlock woolly adelgid. This concurrent development allows *S. camptodromus* eggs to hatch while hemlock woolly adelgid is laying eggs. This synchrony between the development of *S. camptodromus* eggs and the overwintering adelgid suggest this beetle may be a good candidate for the biological control of the hemlock woolly adelgid.

KEY WORDS *Scymnus camptodromus*, egg diapause, temperature, hatch

The hemlock woolly adelgid, *Adelges tsugae* (Annand) (Hemiptera: Adelgidae), is an exotic pest native to Asia and western North America (Havill et al. 2007). Some of the earliest records of the adelgid in eastern North America document it in 1951 near Richmond, VA (Gouger 1971). The genotype of hemlock woolly adelgid present in eastern North America is identical to hemlock woolly adelgid found in southern Honshu, Japan on *Tsuga sieboldii* Carriére (Havill et al. 2006), and was likely brought to the United States on nursery stock (Havill and Montgomery 2008). In the early 1980s, the distribution of this invasive adelgid began to rapidly expand, and populations can now be found in 18 eastern states where it threatens the two native hemlock species (eastern hemlock, *Tsuga canadensis* (L.) Carriére, and Carolina hemlock, *Tsuga caroliniana* Engelmann) indigenous to eastern North America. Hemlock woolly adelgid has caused extensive decline and mortality of hemlock trees in the eastern United States (Orwig and Foster 1998, 2000), likely the result of limited tree resistance and a lack of natural enemies in the introduced range (McClure 1987). This adelgid currently represents the most serious threat to the stability and sustainability of hemlock as a forest resource in eastern North America.

The tools currently available or under development for the management and control of the hemlock woolly adelgid include: chemical control (Cowles et al. 2006), silvicultural prescriptions (Fajvan and Wood 2008), increased host resistance (both naturally occurring [Caswell et al. 2008] and through the development of hybrids [Montgomery et al. 2009]), and classical biological control (Reardon and Onken 2011). In addition, increased regulatory efforts to avoid the movement of infested material into uninfested regions, and increasing public awareness are critical to reducing the spread of hemlock woolly adelgid. Chemical control, while effective in landscape settings and on individual high-value trees, remains both financially and ecologically unsuitable for use at a landscape scale. Development of resistant hemlock hybrids is currently underway (Montgomery et al. 2009), as is development of silvicultural protocols (Fajvan and Wood 2008). Currently, the establishment of a complex of natural enemies from the native range of hemlock woolly adelgid offers an opportunity

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to achieve sustainable long-term control of hemlock woolly adelgid.

Although adelgids have no known parasites, they do have several predators that prey specifically on them (Cheah et al. 2004). Unfortunately, there are no native predators in the eastern United States that attack hemlock woolly adelgid in numbers sufficient to regulate it below damaging thresholds (Montgomery and Lyon 1996, Wallace and Hain 2000). Classical biological control efforts began in the mid-1990s and several hemlock woolly adelgid predators from Asia and the Pacific Northwest have been released into eastern North America (Cheah et al. 2004). The introduced predators currently established in the eastern United States include *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae), imported from Japan and first released in 1995 (McClure et al. 2000), and *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), imported from the northwestern United States and first released in 2003 (Lamb et al. 2006). Two species of *Scymnus* lady beetles in the subgenus *Neopullus* (Coleoptera: Coccinellidae) imported from China, *Scymnus sinuanodulus* Yu and Yao and *Scymnus ningshanensis* Yu and Yao, were first released in 2004 and 2007, respectively, but are not known to be established (Montgomery and Keena 2011). A third species imported from China, *Scymnus* (*Neopullus*) *camptodromus* Yu and Liu, has the broadest geographic range among the species from China (Yunnan and Sichuan Provinces), and is consistently present over a wide range of habitats and hemlock woolly adelgid densities (Montgomery and Keena 2011).

Both the distribution and the unique biology of this species make this beetle a candidate for biological control. The biology of this species differs from the two previously released *Scymnus* species in that the adult beetles begin oviposition about a month after eclosion, and their eggs (laid when adelgids are laying eggs) enter an aestivo-hibernal diapause, and do not hatch until the following spring. The other two species (*S. ningshanensis* and *S. sinuanodulus*) do not lay eggs until the spring following eclosion, and the eggs hatch in ≈10 d (Montgomery and Keena 2011). Egg diapause is uncommon among the Coccinellidae, with the only other record of an egg diapause being from *Scymnus* (*Pullus*) *impexus* (Mulsant), a predator of *Adelges piceae* (Ratzeburg) in Europe (Delucchi 1954).

Diapause is induced in advance of the beginning of the adverse conditions, most often by photoperiod, but changes in such things as food quality/quantity, moisture, and temperature may also play a role in induction. In addition, temperature can regulate the rate of diapause development, and determine how fast postdiapause development occurs (Tauber et al. 1986). In this article, we evaluate whether temperature and/or photoperiod play a role in induction and termination of diapause. We focus primarily on the conditions needed for termination of diapause and the completion of postdiapause development in *S. camptodromus* eggs so that mass rearing methods can be developed and host range testing completed for this predator. The terms associated with diapause used in this article are taken from Koštál (2006).

S. camptodromus was first imported from China into the United States in 1995, and despite importing several hundred specimens over the 3 years, a breeding colony was not successfully established in quarantine. Although it was recognized early on that this lady beetle had an unusual egg diapause (Lu and Montgomery 2000), and preliminary studies indicated that exposure to cold temperatures may break the diapause (C.C., unpublished data), the environmental conditions needed to promote and predict egg development remained undetermined. The reimportation of *S. camptodromus* from 2005 to 2007 made it possible to more thoroughly and systematically examine the regulation of the egg diapause in this species. In these studies we evaluated the effects of temperature and photoperiod on the egg diapause, and determined the temperature requirements for egg hatch. The impact of these findings on the potential for developing this species for release against hemlock woolly adelgid is discussed.

Materials and Methods

Insects. The collection information for the geographic populations of *S. camptodromus* used in these studies is given in Table 1. The *S. camptodromus* adults were transported from China, under permit, to the U.S. Department of Agriculture Forest Service quarantine facility in Ansonia, CT, where these studies were conducted. Voucher specimens of adults were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT.

The geographic source of the eggs was tracked in each study, except for the group of beetles mentioned in the footnote for Table 1 that was from several geographic locations (designated CYS, which was a mixture of the SFF, NBG, MNP, and LJS populations collected in 2005). Eggs used in the first study were either laid by beetles collected and imported in 2005 or by their progeny. All of the adults imported in 2005–2006, and their progeny, were lost in December 2006 when a chamber malfunctioned and they were exposed to >30°C temperatures. The second study was conducted using eggs laid by beetles from new collections made in China from the fall of 2006 to the fall of 2007, while the third study used eggs laid by their progeny. Because the number of adults laying eggs and the number of eggs available from each population varied over time, neither the populations used in each study nor the number of eggs from each population was consistent or balanced.

Obtaining Eggs. *S. camptodromus* prefers to lay eggs in bud scales that have formed a tight curl or in male hemlock cones (old or new), but will also use other concealed locations. The females will lay eggs for several months, if they have hemlock woolly adelgid eggs to feed on, are held at 10–25°C (higher temperatures have not been evaluated), and have adequate places to hide their eggs.

Table 1. Location of the source populations of *Scymnus camptodromus* from China evaluated

Province	County	Site name	Short name	Latitude (north)	Longitude (east)	Altitude (m)	Year: Collection date (no. live adults)
Sichuan	Jinchuan	Sesiman	SFF	31.30°	101.30°	2,784	2005: June 28 (5)[a]
Sichuan	Baoxing	Nibagou	NBG	30.68°	102.68°	2,750–2,790	2005: May (44), Sept. (1)[a] 2007: April 1 (18), May 6 (19)
Sichuan	Danba	Dingguoshan	DGS	30.61°	101.75°	2,890–3,100	2006: Oct. 5 (13), Nov. 5–11 (55) 2007: April 26 (28)
Yunnan	Yulong	Maoniuping	MNP	27.17°	100.26°	3,168–3,170	2005: Sept. 25–28 (15)[a] 2007: April 20 (13), June 13 (7), Nov. 23 (35)
Yunnan	Yulong	Laojunshan	LJS	26.66°	99.81°	2,930–3,027	2005: Sep 23 (3)[a] 2007: April 21–22 (18), May 25 (19), June 11 (12), Nov. 21 (11)
Yunnan	Lanping	Shanshenmiao	LP	26.45°	99.34°	2,780–2,956	2007: May 23 (17), June 10 (17), Nov. 20 (30)

[a] Combined as a single colony before this study; this group referred to as CYS in text.

To obtain the eggs for each study, groups of 10–15 adults were held in white 4.7 liter plastic containers (product T801163, Berry Plastics Corp., Evansville, IN) for the first study and in clear 7.6 liter plastic tubs (product 6308, Rubbermaid, Fairlawn, OH) for the other studies. The lids of each container had an 11 cm circular opening cut in the plastic and a piece of fine mesh cloth was used to cover the mouth of the container before the lid was snapped on over it. Two 5 cm diameter holes were cut in the sides of the plastic tubs and the fine mesh cloth was secured over them using a hot glue gun. A 236 ml plastic cup with a 1.5 cm diameter hole cut in the center was used as a water reservoir. A 2 × 8 cm piece of rolled cotton was wrapped around the ends of 3–4 hemlock woolly adelgid infested branch tips of *T. canadensis* (15 cm long), this was pushed through the lid opening, the cut ends of the branch tip were trimmed under water and the lid with the branch tips was snapped in place on the water reservoir. The foliage in the water reservoir was placed on top of a paper towel in the container, the beetles added, and the lid snapped in place. Branches of infested foliage were collected in the winter and spring when hemlock woolly adelgid is active and then held at 4–5°C with their ends in water (recut every other week) and misted every other day until used. All the studies used eggs laid in the spring when the infested branches were fresh and when ample newly laid hemlock woolly adelgid eggs were available to the adult beetles. The foliage was changed either every other week (study 1) or once a week (studies 2 and 3), and the old foliage was searched for eggs. The eggs were carefully removed from the foliage using a fine damp paint brush and fine forceps under a dissecting microscope and placed in plastic containers.

Laboratory Assays. All studies were conducted in environmental chambers that maintained temperatures to ±1°C of the set points (see below for exact temperatures). Low intensity, broad spectrum florescent lights were used in each chamber. The relative humidity (RH) in the chambers was 80 ± 5% for all studies. The adult females and their eggs while on the foliage were exposed to this humidity, but for the studies the eggs were removed from the foliage and placed in containers so their hatch could be observed. The containers with the eggs were placed on a screen over water in a clear plastic box (water box) to maintain near 100% RH. If mold started to develop on the eggs or filter paper, a 5% bleach solution was used to remove it and help prevent regrowth of the mold.

There were three separate studies that are referred to as studies 1 through 3. In each study, eggs were exposed to 5°C for various numbers of days (0–84 d). When the time at 5°C was completed (for some eggs this was immediately after harvest), eggs were held at a higher temperature (10, 15, or 20°C) until they hatched, were determined to be nonviable, or the study was ended. The day each egg hatched was recorded and the condition of eggs that did not hatch was classified as: 1) darkened, 2) covered with fungus, 3) desiccated, or 4) no change in appearance. Eggs that darkened had signs of partial development while those in the last two classes had no signs of development. Eggs that became covered with fungus early in the studies were likely damaged in removal from the hemlock, while those that grew fungus later in the study often showed some signs of development but did not hatch when expected.

Study 1. In the first study, the eggs were obtained from females held at 20°C and a photoperiod of 16:8 (L:D) h. These conditions were used to mimic a late spring or early summer environmental condition. The eggs were held 3–14 d at 20°C before they were removed and placed individually in cells of a 24-well plate, at which time the plate was moved to 5°C. Each treatment of this study included 34–43 eggs, 22–31 from the 2005 NBG population and 11–12 from the CYS group. There were eight treatments as follows: four different lengths of time that eggs were held at 5°C (7, 28, 56, and 84 d) followed by two incubation temperatures (10 and 15°C) for hatch. The chambers used to hold the eggs had a photoperiod of 12:12 (L:D) h. Eggs were checked twice a week until first hatch, each weekday when a few eggs were hatching, and every day during peak hatch. Eggs were observed for a total of 180 d after being moved to 10 or 15°C.

Study 2. Females collected in China in 2006 and 2007 (Table 1) were held at 15°C and a photoperiod

of 12:12 (L:D) h to obtain eggs for this study. These conditions were meant to mimic early to mid spring environmental conditions. The eggs were removed from the foliage and all the eggs laid over the course of a week by each population were held separately in a 35 × 10 mm petri dish with a 32 mm filter paper in the bottom for 21 d at 15°C, after which the eggs were allocated randomly among the treatments. Each treatment included 29–31 eggs from MNP, 8–9 LP, 5–6 LJS, and 3 each from DGS and NBG (50 total eggs). There were eight treatments as follows: three constant temperatures where eggs were held at either 10, 15, or 20°C, three treatments where the eggs were held for 84 d at 5°C and then moved to either 10, 15, or 20°C for hatch, and two treatments where eggs were held at 5°C for 56 d followed by 10 or 15°C. The photoperiods in the chambers (simultaneously used for other studies that required them) were 12:12 (L:D) h for the 5 and 15°C, and 16:8 (L:D) h for the 10 and 20°C. Eggs were checked twice a week until first hatch, each weekday when only a few eggs were hatching, and daily during peak hatch. The study ended when all eggs had either hatched or did not appear to be viable.

Study 3. Eggs were obtained from the F-1 progeny of the 2007 MNP population reared in the laboratory from females held at 15 ± 1°C and a photoperiod of 16:8 (L:D) h. There were 30 eggs in each of three treatments held at 28, 56, or 84 d at 5°C, and then moved to 10°C for hatch. The photoperiod was 16:8 (L:D) h for all phases of the study to determine if changes in photoperiod (either up or down) that the eggs experienced between the time they were laid and hatched were required for eggs to hatch (i.e., photoperiod was part of the diapause breaking stimulus). Eggs were checked daily during the week and the study was ended after 214 d at 10°C.

Statistical Analysis. Time to hatch was measured as days the eggs were placed at the incubation (post 5°C treatment temperature) or constant temperature (10, 15, or 20°C) and total time to hatch is the total days eggs were held from harvest off the foliage to hatch regardless of temperature (this would be equivalent to time to hatch for constant temperatures). Time to hatch and total time to hatch were analyzed in PROC MIXED (SAS Institute 1999) using Restricted Maximum Likelihood (REML) estimation methods, which provides unbiased estimates of variance in unbalanced designs. The model used time at the 5°C chill period, the temperature at which eggs were incubated after the chill period, and the interaction between the two factors as fixed effects. The source population was included in the model as a random effect. Time to hatch was also compared between studies separately for both the 56 and 84 d at 5°C followed by incubation at 10°C treatments to see if there were any significant differences between the studies that would preclude combining the results for later analyses. The models used for these comparisons treated different studies as a fixed effect and the source population as a random effect. Because the studies could be combined, the response of the MNP and NBG populations were compared for the 84 d at 5°C followed by incubation at 10°C treatment using population as a fixed effect and study as a random effect. Differences among means were determined using the least squared means test with $\alpha = 0.05$ and a Bonferroni correction (SAS Institute 1999). Percentage hatch was compared using pair wise Pearson's χ^2 analysis between treatments (Statistix 2003).

All of the data for the three studies was also combined for the treatments that involved days held at 5°C (D_5) followed by incubation at 10°C to evaluate how the developmental rate at 10°C (1/d to hatch at 10°C, DR) changes with increasing time at 5°C. The relationship between developmental rate and time at 5°C was described using an exponential function,

$$DR = a * \exp^{(b * D_5)} \quad [1]$$

in which a and b are the developmental rate when 0 d are spent at 5°C and the percent increase in developmental rate for each day at 5°C, respectively (PROC NLIN and Marquardt convergence method; SAS Institute 1999).

The critical minimum temperature for development (T_{crit}) was calculated using all eggs held first for 84 d at 5°C, then at 10–15°C until hatch. These data were used because hatch rates for eggs held longer at higher temperatures (shorter at five degrees) demonstrated extended development times and lower hatch rates, indicating these temperatures exceed the optimal development temperature for *S. camptodromus* eggs. The critical temperature was determined analytically by solving for T_{crit} in the following equation

$$[(D_5 * (5 - T_{crit})) + (D_{10} * (10 - T_{crit}))] = [(D_5 * (5 - T_{crit})) + (D_{15} * (15 - T_{crit}))] \quad [2]$$

Based on the time spent at both 5°C and the higher temperature used for rearing. Note that the equation reduces simply to:

$$[D_{10} * (10 - T_{crit})] = [D_{15} * (15 - T_{crit})] \quad [3]$$

when contant times at 5°C are used.

Where D_5 is the number of days spent at 5°C, D_{10} and D_{15} are the mean number of days spent at 10 and 15°C, respectively. Critical temperature was used to calculate the total number of Heating Degree Days (HDD) required for egg hatch. HDD are calculated by summing the degrees above the critical temperature accumulated daily.

Results

Effects of Temperature. *Study 1.* The number of days eggs were held at 5°C ($F = 48.86$; df = 3, 145; $P < 0.0001$), the incubation temperature ($F = 35.06$; df = 1, 145; $P < 0.0001$), and an interaction between the two ($F = 13.74$; df = 3, 145; $P < 0.0001$) all had significant impacts on the time it took eggs to hatch at the incubation temperature. Average days to hatch once eggs were moved to 10°C decreased significantly as the length of time eggs were exposed to 5°C increased (Table 2). The only significant difference in days to hatch for eggs moved to 15°C was that those held at

Table 2. Mean time to hatch (days ± SE) at the incubation temperature and mean total time to hatch at treatment temperatures (days ± SE), either 5°C followed by 10 to 20°C or constant temperatures of 10 to 20°C, for *Scymnus camptodromus* eggs

Study[a]	Time at 5°C (days)	Incubation temperature (°C)	*n*	Mean time to hatch (d)[b]	Mean total time to hatch (d)[b]
1	7	10	23	128.9 ± 8.2a	133.0 ± 9.5c
	28	10	28	091.1 ± 7.9b	121.1 ± 9.4bc
	56	10	33	065.7 ± 7.9c	118.8 ± 9.3bc
	84	10	21	032.8 ± 8.3d	113.6 ± 9.6b
	7	15	4	055.5 ± 11.9cd	058.5 ± 12.9a
	28	15	6	075.4 ± 10.4bc	103.7 ± 11.7b
	56	15	18	065.0 ± 8.4c	118.5 ± 9.8bc
	84	15	23	030.3 ± 8.3d	111.4 ± 9.5b
2	0	10	36	224.5 ± 6.1a	224.5 ± 6.1b
	0	15	0	No hatch	No hatch
	0	20	2	102.0 ± 19.6b	102.0 ± 19.6a
	56	10	40	057.2 ± 5.9b	113.2 ± 5.9a
	84	10	38	033.3 ± 6.1cd	117.3 ± 6.1a
	56	15	8	062.3 ± 10.6bc	118.3 ± 10.6a
	84	15	12	016.4 ± 8.8d	100.4 ± 8.8a
	84	20	3	020.4 ± 16.1cd	104.4 ± 16.1a
3	28	10	16	115.7 ± 6.1a	143.7 ± 6.1b
	56	10	22	059.3 ± 5.2b	115.3 ± 5.2a
	84	10	27	037.1 ± 4.7c	121.1 ± 4.7a

[a] In study 1, eggs were laid at 20 ± 1°C and a photoperiod of 16:8 (L:D) h and the photoperiod for the treatment temperatures was 12:12 (L:D) h. In study 2, eggs were laid at 15 ± 1°C and a photoperiod of 12:12 (L:D) h and the photoperiod for the treatment temperatures was 12:12 (L:D) h for the 5 and 15°C, and 16:8 (L:D) h for the 10 and 20°C. In study 3, eggs were laid at 15 ± 1°C and a photoperiod of 12:12 (L:D) h was used in the 5, 10, and 15°C chambers.

[b] Values followed by the same letter within each study are not significantly different.

5°C for 84 d on average hatched faster than those held for 28 or 56 d. The mean days to hatch for the 10 and 15°C treatments were not significantly different, except when the eggs were held for only 7 d at 5°C.

The number of days eggs were held at 5°C ($F = 5.02$; df = 3, 145; $P = 0.0024$), the incubation temperature ($F = 36.17$; df = 1, 145; $P < 0.0001$) and an interaction between the two ($F = 14.14$; df = 3, 145; $P < 0.0001$) all had significant impacts on the total time it took eggs to hatch (days at 5°C plus time at the incubation temperature). Eggs held for 7 d at 5°C followed by incubation at 15°C took on average half the time to hatch compared with all the other treatments (Table 2). Eggs held for 7 d at 5°C followed by incubation at 10°C took significantly longer to hatch than eggs in several of the other treatments.

The percentage hatch of eggs increased as the time held at 5°C increased, for those held 84 d at 5°C and then incubated at 10°C that had a lower percentage hatch than expected based on the hatch of the other treatments (Table 3). Eggs moved to 10°C had higher hatch than those moved to 15°C, except for those held 84 d at 5°C. The status of eggs that did not hatch at the end of 180 d is given in Table 3. In general, more eggs incubated at 15°C than at 10°C darkened, which happened after signs of partial development were evident. The longer the time spent at 15°C the more eggs developed fungus, desiccated, or remained visibly unchanged from the time they started the study.

Study 2. The number of days eggs were held at 5°C (0, 56, or 84; $F = 80.92$, df = 2, 128, $P < 0.0001$), the incubation temperature (10, 15 or 20°C ; $F = 14.09$, df = 2, 128, $P < 0.0001$), and an interaction between the two ($F = 10.93$; df = 2, 128; $P < 0.0001$) all had significant impacts on the time it took eggs to hatch at the incubation or constant temperature. Average days to hatch once eggs were moved to the incubation temperature decreased significantly as the length of

Table 3. Percentages of eggs hatched, missing, and condition of unhatched *Scymnus camptodromus* eggs

Study	Time at 5°C (days)	Incubation temperature (°C)	Total eggs used	Hatched eggs (%)	Missing eggs (%)[b]	Condition of unhatched eggs (% of all eggs)[a]			
						Partial development	Damaged egg or dead embryo	No visible development	
						Darkened	Growing fungus	Unchanged when study ended	Desiccated
1	7	10	41	56.1bc	2.4	2.4	0.0	17.1	22.0
	28	10	42	66.7b	0.0	9.5	2.4	21.4	0.0
	56	10	38	86.8a	0.0	5.3	0.0	5.3	2.6
	84	10	34	61.8b	0.0	2.9	5.9	23.5	5.9
	7	15	43	9.3e	0.0	18.6	20.9	34.9	16.3
	28	15	34	17.6d	0.0	5.9	20.6	38.2	17.6
	56	15	42	42.9c	0.0	33.3	16.7	7.1	0.0
	84	15	41	56.1bc	7.3	9.8	2.4	19.5	4.9
2	0	10	50	72.0a	2.0	8.0	14.0	0.0	4.0
	0	15	50	0.0c	8.0	6.0	16.0	0.0	70.0
	0	20	50	4.0c	2.0	26.0	22.0	0.0	46.0
	56	10	50	80.0a	0.0	2.0	8.0	0.0	10.0
	84	10	50	76.0a	2.0	4.0	6.0	0.0	12.0
	56	15	50	16.0b	0.0	44.0	10.0	0.0	30.0
	84	15	50	24.0b	6.0	46.0	12.0	0.0	12.0
	84	20	50	6.0c	16.0	42.0	14.0	0.0	22.0
3	28	10	30	53.3c	0.0	10.0	3.3	26.7	6.7
	56	10	30	73.4b	0.0	10.0	3.3	0.0	13.3
	84	10	30	90.1a	0.0	3.3	3.3	0.0	3.3

[a] A few eggs disappeared from the holding containers during the studies. Some eggs were accidently lost when opening and closing containers and others were likely eaten by siblings that hatched and were not removed quickly enough.

[b] Percentages of hatched eggs followed by the same letter in each study are not significantly different.

time eggs were exposed to 5°C increased (Table 2). Mean time to hatch did not vary with incubation temperature for eggs exposed to 56 or 84 d at 5°C. Average days to hatch at a constant 10°C was significantly longer than at 20°C, but the eggs that hatched at 20°C did so at about the same time that eggs held at 10°C began hatching.

The number of days eggs were held at 5°C ($F = 22.62$; df = 2, 128; $P < 0.0001$), the incubation temperature ($F = 14.09$; df = 2, 128; $P < 0.0001$), and an interaction between the two ($F = 10.93$; df = 2, 128; $P < 0.0001$) all had significant impacts on the total time it took eggs to hatch (days at 5°C and at the incubation or at the constant temperature). Average total days to hatch for eggs held a constant 10°C was nearly twice that of eggs in all other treatments.

Only two eggs (4%) hatched when held at a constant 20°C, three eggs (6%) hatched when held for 84 d at 5°C then incubated at 20°C, and no eggs hatched at a constant 15°C (Table 3). Percentage hatch for eggs incubated at 10 or 15°C did not vary with the number of days spent at 5°C. Only 6% of the eggs held at a constant 15°C appeared to develop partially then darkened while >70% showed no signs of development and most eventually desiccated (Table 3). When eggs were held at 5°C before incubation at 15 or 20°C, >40% of the eggs that did not hatch darkened, after either partial or complete development of the embryo (Table 3).

Study 3. The number of days eggs were held at 5°C had a significant impact on the time it took eggs to hatch at 10°C ($F = 51.88$; df = 2, 62; $P < 0.0001$) and on the total time it took eggs to hatch (time at 5 and 10°C combined; $F = 6.67$, df = 2, 62, $P = 0.0024$). Average days to hatch once eggs were moved to 10°C decreased significantly as the length of time eggs were exposed to 5°C increased (Table 2). Total time to hatch for eggs that received only 28 d at 5°C was significantly longer than for eggs held for 56 or 84 d at 5°C (Table 2). Percentage hatch increased as the time the eggs were held at 5°C increased. Only eight eggs from the 28 d treatment had not hatched or been removed because of darkening, mold or desiccation at the time the study was ended (Table 3).

Variation Among Geographic Sources. When the two egg temperature treatments that were the same across all three studies (56 or 84 d at 5°C followed by incubation at 10°C) were compared separately (using geographic source as a random effect), there was no significant difference between the studies (56 d at 5°C, $F = 1.03$, df = 2, 87, $P = 0.3631$ and 84 d at 5°C, $F = 0.43$, df = 2, 78, $P = 0.6552$). Of the geographic populations, the NBG and MNP had sufficient numbers to compare their response to temperature, at both 56 and 84 d at 5°C followed by incubation at 10°C. The NBG population on average hatched significantly faster than the MNP population (56 d at 5°C, $F = 7.15$, df = 1, 69, $P = 0.0094$ and 84 d at 5°C, $F = 0.43$, df = 1, 65, $P < 0.0001$) and had more intra-population variation in response to temperature than did the MNP population (Fig. 1).

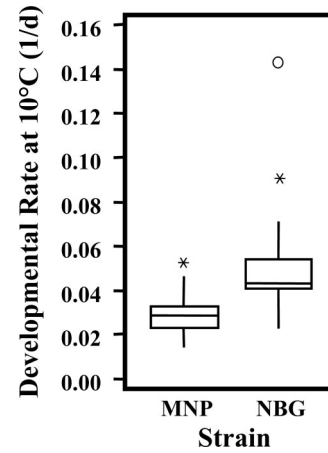

Fig. 1. Box plot comparison of the developmental rate at 10°C (1/d) for *Scymnus camptodromus* eggs from the MNP and NBG geographic populations held for 84 d at 5°C. Values for eggs from all studies were combined.

Effects of Photoperiod on Egg Hatch. There were no apparent effects of the photoperiod the eggs were laid at, subsequently held at (either during chill or incubation), or the direction of change in the photoperiod over time (increase, decrease, or stay the same) on the percentage of eggs that hatched or timing of hatch. All three studies had eggs that were exposed to 5°C for 56 or 84 d and then incubated at 10°C and the percentage hatch and timing of hatch were consistent despite the photoperiods used. The eggs that were part of these two treatments in study 1 experienced a decline in the number of hours of light (16 when laid and 12 thereafter), in study 2 they experienced an increase in the hours of light (from 12 to 16) and in study 3 there was no change in photoperiod (16 h of light). The females that laid eggs had experienced an increase in temperature (from 10 to 15 or 20°C) and either a decrease (study 2, 16–12) or increase (studies 1 and 3, 12–16) in the number of hours of light a month or two before the eggs were laid, primarily because food was limited earlier and lower temperatures increase survival under those conditions.

Egg Development Models. In nearly all of the treatments, eggs began to hatch between 70 and 100 d after they were first placed at the initial 5°C temperature (total time to hatch; Fig. 2). No eggs hatched before they were placed at one of the higher temperatures. Shortest total time to hatch occurred with eggs held only 7 d at 5°C and then moved to 15°C, but only 3 (9%) of these eggs ever hatched. Except for this anomaly, eggs held longer at 5°C, hatched sooner when moved to a higher temperature. The second temperature had a greater influence on the total percentage of egg hatch than on average time of hatch of the eggs. The apparent developmental rate at 10°C for eggs increased exponentially ($DR = 0.00534 \pm 0.000625 * \exp^{(0.0219 \pm 0.00153 * D_5)}$; $r^2 = 0.5537$; $F = 746.07$; df = 2, 282; $P < 0.0001$) as time held at 5°C increased (Fig. 3).

We calculated the T_{crit} using the eggs that spent the majority of their developmental period at 5°C (84 d)

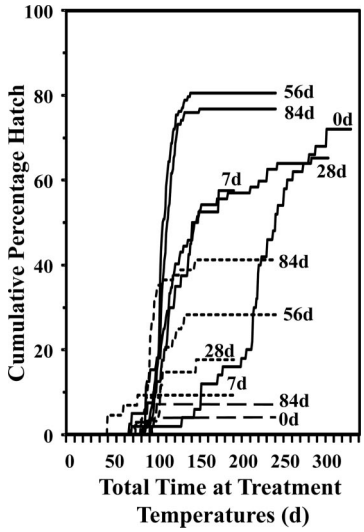

Fig. 2. Cumulative percentage hatch of *Scymnus camptodromus* eggs over the total time held at treatment temperatures (either 5°C followed by 10–20°C or constant temperatures of 10–20°C). Incubation temperatures are shown as solid lines for 10°C, short dashed lines for 15°C, and long dashed lines for 20°C. The number of days at 5°C before incubation is shown as a label associated with each line, constant temperatures being indicated as 0 d.

using equation 3 listed in the methods. This resulted in an estimated T_{crit} of 2°C, indicating eggs held at 5°C break diapause, and are likely able to proceed with development without exposure to higher temperatures. Using this T_{crit}, the mean estimated number of days required for egg development is ≈525 HDD; however, this includes some time spent at 10 degrees and given the apparent increase in developmental rate with increased incubation time at 5°C, the calculated HDD required for development may be an overestimate.

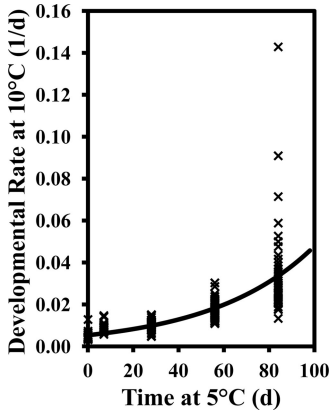

Fig. 3. Developmental rate at 10°C (1/d) for *Scymnus camptodromus* eggs held for 0, 7, 28, 56, or 84 d at 5°C. Values for eggs from all studies and geographic populations were combined and an exponential function (developmental rate = $0.005*e^{0.02*\text{days at } 5°C}$; $r^2 = 0.55$) was fitted to the data (solid line).

Discussion

All *S. camptodromus* eggs appeared to go into diapause when laid (no apparent signs of embryo development) at ≥15°C because 103 d was the shortest time to hatch for any egg held at constant temperatures >15°C and we assume that nondispausing eggs would hatch in the ≈10 d that it takes eggs of other *Scymnus* species to hatch (Montgomery and Keena 2011). Because only 4% of the eggs that received no exposure to temperatures <15°C hatched, we conclude that although not needed for all eggs, the majority of eggs required lower temperatures for diapause termination and postdiapause development. The fact that few eggs held at a constant 15 or 20°C showed any visible signs of possible embryo development further substantiates that warmer temperatures act to maintain diapause until the eggs experience the required cold temperatures. Females can lay eggs at temperatures <15°C [both in the laboratory at 10°C (M.K., unpublished data) and in Nibago, China, during April when the temperature averages 7°C (M.E.M., unpublished data)]. Therefore, it is possible that these eggs may not go through a diapause if kept at those lower temperatures, but this has not been tested. More work needs to be done to determine if *S. camptodromus* eggs go through a diapause regardless of the temperature at which they are laid.

Photoperiod at the time the eggs were laid or subsequent changes in it did not appear to affect time to hatch or percentage hatch when treatments that were the same across the three studies were compared. This indicates that photoperiod is not involved in breaking diapause and that it likely does not function as a cue for eggs to enter diapause. The possible effect of moisture on egg diapause was not investigated because the eggs were all held under high humidity conditions regardless of temperature.

The locations in China where *S. camptodromus* is found generally are at more southern latitudes and higher altitudes than the target release areas in the eastern United States. Winter temperatures in the target areas and in the locations in China where the beetles are found are similar, but the summer temperatures are lower in China (Montgomery and Keena 2011). Locations where *S. camptodromus* was found in Yunnan generally have warmer winters than those in Sichuan; at Nibagou, the coldest site in Sichuan, the average monthly temperatures from November to March are 4–7°C cooler than in Maoniuping in Yunnan, but average temperatures are similar for the rest of the year (hourly temperatures were recorded at each location for 2–3 yr periods between 2000–2006, M.E.M.). These temperature differences affect when hemlock woolly adelgid eggs are present at each of these sites, December to April in Yunnan and January to June (a few even in the summer) in Sichuan (Montgomery and Keena 2011). These temperature differences between Nibagou and Maoniuping, combined with the timing of when hemlock woolly adelgid eggs are available, explain why *S. camptodromus* eggs from Nibagou would be adapted to hatch faster than those

from Maoniuping as was seen in the studies described here. This also shows that *S. camptodromus* is adapted to develop and lay eggs at cooler temperatures and can adapt its phenology to match both that of the local climate and hemlock woolly adelgid.

Our results suggest that *S. camptodromus* has a propensity to be continuously variable in diapause length and that the species may use this as a means to adapt to the natural fluctuations in climate that affect the timing of prey availability. This is not uncommon in invertebrates and has the advantage of bet-hedging to avoid conditions that could result in all eggs hatching when there is no food for the larvae to eat (Tauber et al. 1986, Danks 1987). Low temperatures are better suited for egg development, with exposure as low as 10°C leading to a reduction in development rate. This was evidenced by increasing percent hatch at 10°C as the time held at 5°C increased, and by the much larger range of days to hatch for eggs held at a constant 10°C than for any other treatment. Exposure to temperatures near 15°C reduced the rate of development further, however, this reduction in development rate was limited (when compared with the eggs reared at 5, then 10°C), suggesting that the plateau (range, between optimum, and lowest deleterious temperatures) in the temperature/development rate curve for this species occurs within this range. This reduced effect of temperatures near the threshold has been documented for other beetle eggs such as, of *Diabrotica barberi* Smith and Lawrence (Colepotera: Chrysomelidae) (Fisher et al. 1994).

An estimate of the upper threshold for accumulating the chill units necessary to complete diapause development was not possible using these data because we do not know exactly when diapause ends and postdiapause development begins. Study 3 included a treatment where *S. camptodromus* eggs were held at constant 5°C but a chamber malfunction exposed these eggs to higher temperatures (at day 74, the temperature rose from 5 to 22°C in a 4 h period, and at day 107, the eggs experienced temperatures that fluctuated between 5 and 11°C for 3 d) and then they were returned to 5°C. The eggs in this failed treatment began hatching at day 120 (and continued to hatch over the next 69 d) after their initial placement at 5°C. This suggests that very few heat units were needed for postdiapause development, that eggs completed diapause and hatched while held at 5°C, and that diapause was terminated by ≈100 d, which supports a T_{crit} below 5°C. This also suggests that the low temperature threshold varies with the age of the embryo as suggested previously by Tauber et al. (1986). Such changes may be mediated by a loss or transformation of a chemical at cool temperatures that the female parent puts in the egg that causes embryogenesis to stop until the token stimulus is received, as occurs in some other insect eggs (*Bombyx mori* (L.), e.g., Ti et al. 2004).

In the Eastern United States, decreasing temperatures in the late fall or early winter begin decreasing the intensity of the diapause (physiogenesis proceeds) in *S. camptodromus* eggs. Physisogensis is very slow at that time of year because the prevailing temperatures are moderate, and it accelerates as temperatures approach 5°C. More work is needed to determine if there is a minimum threshold and total chill units needed to terminate diapause. Once diapause is terminated, *S. camptodromus* eggs can begin postdiapause development (morphogenesis) at temperatures as low as 2°C and the speed of development slowly increases with increasing temperature as evidenced by the similarity of time to hatch at 10 and 15°C when eggs had previously received 56 or 84 d at 5°C (enough for most eggs to complete diapause development). This produces a development strategy functionally similar to other insects that enter a postdiapause quiescence after diapause during part of the year (Tauber et al. 1986). This means that physiogenesis and morphogenesis occurred simultaneously in different individual eggs at temperatures ≤10°C because of genetic variability between individuals in diapause intensity or morphogenesis rates. At higher temperatures (e.g., 20°C incubation after 84 d at 5°C) fewer eggs either completed diapause because of insufficient accumulation of chill or more likely because the temperature was too high to complete postdiapause development and hatch normally.

The majority of Coleoptera become quiescent in the adult stage, either during the summer or winter, and among aphidophagous species scarcity of prey is the main factor that induces the diapause (Masaki 1980). Delucchi (1961) indicates that *S. impexus* hibernates in bark crevices during the winter and are scarce during the hot summer months, when food is scarce and *S. camptodromus* seems to follow a similar pattern in China. Only ≈2% of Coleoptera have an egg diapause (all aestival; Masaki 1980). There is little information on the thermal requirements for coccinellid eggs in general, but for those that have been studied, the lower developmental threshold for morphogenesis ranges between 5.6 and 12.2°C (Frazer and McGregor 1992, Honěk and Kocourck 1988). The egg aestivo-hibernal diapause of *S. camptodromus* allows it to synchronize with the phenology and availability of its host. There are two apterous generations of hemlock woolly adelgid that complete development on eastern hemlocks: progrediens from February/March to June/July, and sistens, from June/July through the following March /April (some latitudinal and yearly variation in timing; Salom et al. 2002, Gray and Salom 1996, McClure 1989). The first instars of the sistens generation go through an aestival diapause that ends in the fall then proceed to develop slowly, mature anywhere from January in the South to March in the North depending on temperatures, and then begin laying eggs (Gray and Salom 1996). It is the eggs of both adelgid generations that *S. camptodromus* adults need to feed on to lay eggs (that will not hatch until the next calendar year) and the larvae feed on to complete their development (likely more the sistens than the progrediens). Therefore, the fact that *S. camptodromus* eggs go through an aestivo-hibernal diapause and have the ability to proceed with postdiapause development at winter temperatures allows them to time their hatch when adelgid eggs are pres-

ent for them to feed. This similar control of phenology based on temperature between *S. camptodromus* and its host suggests that it should be able to establish and synchronize well with its host. Permission to release this species from quarantine was obtained at the same time as it was for the other two *Scymnus* (*Neopullus*) species imported from China for hemlock woolly adelgid biological control. Work on assessing its host preferences still remains to be done and a method to rear larger numbers needs to be developed before field evaluations can be done.

Acknowledgments

We thank Nathan Havill and Kelli Hoover for their critical review of this article, two anonymous reviewers for suggestions that made this a better article. Jason Biroscak, Rebecca Lomanchinsky, Paul Moore, Vicente Sanchez, and Alice Vandel provided technical assistance. We also thank Wenhua Lu, Li Li, Jianhua Zhou, and Gouyue Yu for obtaining the adult lady beetles to start the colonies.

References Cited

Caswell, T., R. Casagrande, B. Maynard, and E. Preisser. 2008. Production and evaluation of eastern hemlocks potentially resistant to the hemlock woolly adelgid, pp. 124–134. *In* B. Onken and R. Reardon (compilers), Fourth Symposium on Hemlock Woolly Adelgid in the Eastern United States, 12–14 February 2008, Hartford, CT. FHTET-2008-01. U. S. Department of Agriculture, Forest Service, Morgantown, WV.

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

Cowles, R. S., M. E. Montgomery, and C.A.S.-J. Cheah. 2006. Activity and residues of imidacloprid applied to soil and tree trunks to control hemlock woolly adelgid (Hemiptera: Adelgidae) in forests. J. Econ. Entomol. 99: 1258–1267.

Danks, H. V. 1987. Insect dormancy: an ecological perspective. Biological survey of Canada, Series No. 1. Biol. Sur. Can. (Terrestrial Arthropods), Ottawa, Canada.

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.

Fajvan, M. A., and P. B. Wood. 2008. The role of silvicultural thinning in eastern forests threatened by hemlock woolly adelgid (*Adelges tsugae*), pp. 247–256. *In* R. L. Deal (tech. ed.), Integrated Restoration of Forested Ecosystems to Achieve Multiresource Benefits: Proceedings of the 2007 National Silviculture Workshop, 7–10 May 2007, Ketchikan, AK. Gen. Tech. Rep. PNW-733. USDA Forest Service, Portland, OR.

Fisher, J. R., J. J. Jackson, and A. C. Lew. 1994. Temperature and diapause development in the egg of *Diabrotica barberi* (Coleoptera: Chrysomelidae). Environ. Entomol. 23: 464–471.

Frazer, B. D., and R. R. McGregor. 1992. Temperature-dependent survival and hatching rate of eggs of seven species of Coccinellidae. Can. Entomol. 124: 305–312.

Gray, D. R., and S. M. Salom. 1996. Biology of the hemlock woolly adelgid in the southern Appalachians, pp. 26–35. *In* S. M. Salom, T. C. Tigner, and R. C. Readon (eds.), Proceedings of the First Hemlock Woolly Adelgid Review. FHTET 96–10. USDA Forest Service, Morgantown, WV.

Gouger, R. J. 1971. Control of *Adelges tsugae* on hemlock in Pennsylvania. Sci. Tree Topics 3: 9.

Havill, N. P., R. Foottit, and C. von Dohlen. 2007. Evolution of host specialization in the Adelgidae (Insecta: Hemiptera) inferred from molecular phylogenetics. Mol. Phylogenet. Evol. 44: 357–370.

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: 2–9.

Havill, N. P., M. E. Montgomery, G. Yu, S. Shiyake, and A. Caccone. 2006. Mitochondrial DNA from hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae), suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. Ann. Entomol. Soc. 99: 195–203.

Honěk, A., and F. Kocourek. 1988. Thermal requirements for development of aphidophagous Coccinellidae (Coleoptera), Chrysophidae, Hemerobiidae (Neuroptera), and Syrphidae (Diptera): some general trends. Oecologia 76: 455–460.

Koštál, V. 2006. Eco-physiological phases of insect diapause. J. Insect Physiol. 52: 113–127.

Lamb, A. B., S. M. Salom, L. T. Kok, and D. L. Mausel. 2006. Combined field release of *Laricobius nigrinus* (Coleoptera: Derodontidae), a predator of the hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), in Virginia. Can. J. For. Res. 36: 369–375.

Lu, W., and M. E. Montgomery. 2000. Comparative biology of three *Scymnus* lady beetles (Coleoptera: Coccinellidae): predators of *Adelges tsugae* (Homoptera: Adelgidae), pp. 188. *In* K. A. McManus, K. S. Shields, and D. R. Souto (eds.), Symposium on Sustainable Management of Hemlock Ecosystems in Eastern North America, Durham, NH, 22–24 June 1999. USDA Forest Service, Northeastern Research Station, Newtown Square, PA.

Masaki, S. 1980. Summer diapause. Annu. Rev. Entomol. 25: 1–25.

McClure, M. S. 1987. Biology and control of hemlock woolly adelgid. Bull. Conn. Agric. Exp. Stn. 851: 1–9.

McClure, M. S. 1989. Evidence of a polymorphic life cycle in the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). Environ. Entomol. 19: 36–43.

McClure, M. S., C.A.S.-J. Cheah, and T. C Tigner. 2000. Is *Pseudoscymnus tsugae* the solution to the hemlock woolly adelgid problem?: an early perspective, pp. 89–96. *In* K. McManus, K. Shields, and D. Souto (eds.), Proceedings, Symposium on Sustainable Management of Hemlock Ecosystems in Eastern North America, 22–24 June 1999, Durham, NH. Gen. Tech. Rep. NE-267. USDA Forest Service, Newtown Square, PA.

Montgomery, M. E., S. E. Bentz, and R. T. Olsen. 2009. Evaluation of Hemlock (Tsuga) species and hybrids for resistance to *Adelges tsugae* (Hemiptera: Adelgidae) using artificial infestation. J. Econ. Entomol. 102: 1247–1254.

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* Proceedings, First Hemlock Woolly Adelgid

Review. FHTET 96–10. USDA Forest Service, Morgantown, WV.

Orwig, D., and D. Foster. 1998. Forest response to the introduced hemlock woolly adelgid in southern New England, USA. J. Torrey Bot. Soc. 125: 60–73.

Orwig, D. A., and D. R. Foster. 2000. Stand, landscape, and ecosystem analyses of hemlock woolly adelgid outbreaks in Southern New England: an overview, pp. 123–125. *In* K. A. McManus, K. S. Shields, and D. T. Souto (eds.), Proceedings: Symposium on Sustainable Management of Hemlock Ecosystems in Eastern North America, 22–24 June 1999, Durham, NH. Gen. Tech. Rep. NE-267. USDA Forest Service, Radnor, PA.

Reardon, R., and Onken, B., (eds.). 2011. Implementation and status of biological control of the hemlock woolly adelgid. USDA Forest Service, FHTET 2011-04.

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

SAS Institute. 1989. SAS/STAT user's guide, version 6, 4th ed., vol. 2. SAS Institute, Cary, NC.

Statistix. 2003. Statistix for Windows user's manual. Analytical Software, Tallahassee, FL.

Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. Seasonal Adaptations of Insects. Oxford University Press, New York, NY.

Ti, X., N. Tuzuki, N. Tani, E. Morigami, M. Isobe, and H. Kai. 2004. Demarcation of diapause development by cold and its relation to time-interval activation of TIME-ATPase in eggs of the silkworm, *Bombyx mori.* J. Insect Physiol. 50: 1053–1064.

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.

Received 23 July 2012; accepted 19 September 2012.