Life History and Development of *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae), a New Predator of the Hemlock Woolly Adelgid (Homoptera: Adelgidae)

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**ABSTRACT** The life history and development of the coccinellid *Pseudoscymnus tsugae* sp. nov. Sasaji & McClure from Japan is described. Both larva and adult feed on all stages of the hemlock woolly adelgid, *Adelges tsugae* Annand, a serious pest of eastern and Carolina hemlock in eastern United States. There are 4 larval instars, a prepupa, and pupa. Total development time from hatch to adult emergence required 29.8 d at 20°C and 17.9 d at 25°C. Incubation time was 10.3 d at 20°C and 6.7 d at 25°C. At 14–21.5°C, males matured at 30 d and females at 31.8 d with a preoviposition period of ≈35 d. At 25°C, males matured at 18.8 d, whereas the female preoviposition period was shortened to 22.4 d. In the laboratory, mean longevity was 162.9 d and 125.6 d for females and males, respectively, with individuals of both sexes that lived >300 d. The mean lifetime fecundity was 279.9 eggs with a maximum of 513 eggs over a mean oviposition period of 14 wk. The mean sex ratio in the laboratory is ≈1:1. *P. tsugae* displays facultative diapause and is capable of producing successive generations in the laboratory when its prey is available, indicating its excellent potential for mass rearing as a biological control agent of *A. tsugae.*

**KEY WORDS** *Adelges tsugae, Pseudoscymnus tsugae, Coccinellidae, Tsuga species,* biological control, predator

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The hemlock woolly adelgid, *Adelges tsugae* Annand, is one of the more important forest and urban pests of the northeastern and mid-Atlantic regions of United States. Susceptible species include the eastern hemlock, *Tsuga canadensis* Carriere, and the Carolina hemlock, *T. caroliniana* Engelmann. In eastern United States, *A. tsugae* is believed to have been first introduced into Virginia in the 1950s and has since spread north and southward into North Carolina, West Virginia, Maryland, Delaware, Pennsylvania, New Jersey, New York, Connecticut, Rhode Island, and Massachusetts. *A. tsugae* infestations on eastern or Carolina hemlock are generally fatal to the tree, with mortality usually occurring between 4 and 6 yr (McClure 1991). There is no effective method of controlling *A. tsugae* in forested areas because the use of chemicals is not feasible and there are no effective indigenous natural enemies to combat the adelgid. Two potentially important natural enemies of *A. tsugae* that help regulate adelgid populations in Japan were collected (McClure 1995a): an oribatid mite, *Diapterobates humeralis* Hermann (McClure 1995b), and a new species of coccinellid, *Pseudoscymnus tsugae* (Sasaji and McClure 1997). Imported shipments of coccinellid larvae and eggs were reared under quarantine laboratory conditions, studied, and a permit for release was obtained from APHIS-PPQ in 1996. The tribe Scymnini, to which *P. tsugae* belongs, is not well known because of its small size and difficult identification although their potential for biological control is recognized (Fang and Gordon 1986). Only a handful of studies have documented the life history of members of this important group; and in the past, several attempts have been made to introduce scymnids for biological control in North America with minor and limited success (Gordon 1976). In this article, we seek to document the life cycle, development, and reproductive biology of this promising new coccinellid predator as part of the continuing evaluation of its potential for biological control of *A. tsugae.*

**Materials and Methods**

**Life Cycle and Morphology of Life Stages.** Observations on the life cycle of *P. tsugae* were made on individuals reared from egg to adult on *A. tsugae* contained in Falcon polystyrene petri dishes (10 by 1.5 cm) lined with Whatman No. 2 filter paper. Eggs of *P. tsugae* were first located in hemlock tips then transferred individually with a fine paintbrush to twigs of hemlock infested with *A. tsugae.* Hatch and development from egg to adult were followed through daily observations using a binocular dissecting microscope to record the number of molts per larva. Measurements were also made with an eyepiece micrometer of each stage and morphological characteristics noted.

**Egg Hatch and Larval Development at Different Temperatures.** The experiments were carried out in an incubator at 20 and 25°C at a photoperiod of 16:8 (L:D) h. Mated females were confined individually in
Fisher polyethylene 237 ml containers with infested hemlock tips for 24 h. At the end of the period, eggs were removed and transferred to freshly-cut, adelgid-infested hemlock tips (4-5 cm long) and kept in petri dishes (10 by 1.5 cm) lined with filter paper. Each egg was monitored daily for hatch with 26 replicates at 20°C and 53 replicates at 25°C.

Larval development was studied using eggs retrieved from colony cages placed in individual polyethylene 237 ml containers, lined with filter paper, and ventilated with holes (1.5 cm in diameter) bored into the lids and covered by fine mesh. The cut-ends of infested hemlock tips were wrapped in cotton and moistened lightly every 2 d. Because all stages of the adelgid are consumed by adult and larvae (Cheah and McClure 1996), the 1st phase of the experiment investigated if prey stage influenced development time. Developing larvae were transferred to either fresh supplies of adelgid ovisacs (n = 17 at 20°C) or developing adelgid nymphs (N1–N4) on fresh tips (n = 32 at 20°C) every 2 or 3 d. Development time from hatch to adult emergence was recorded in days. The 2nd phase of the experiment measured the duration of each stage at different temperatures in the same way. Tips were examined twice daily, in the morning and late afternoon, except for weekends when only 1 afternoon examination was made on Saturday. The duration in days of each instar, prepupa, and pupa at the different temperatures was recorded (n = 28–41). Replicates that did not successfully develop to adult emergence were not included in the calculation of means and standard deviations. Larval development from egg to adult was also repeated at 25°C on a prey diet of adelgid ovisacs and crawlers (n = 41).

**Mating Behavior, Fecundity, Longevity, and Sex Ratio.** Thirty-seven pairs of newly emerged adult *P. tsugae* were maintained on adelgid-infested hemlock tips kept in transparent round centrifuge tubes (10 by 3.7 cm) with ventilated caps at room temperature (20–23°C:12-16°C [L:D]) under fluorescent lights. Observations on the activity of each pair were made 4 times a day to record the 1st instance of mating (measurement of reproductive maturity) and the initiation of oviposition (measurement of the precopulation period). This frequency of observation was sufficient as copulation lasts for at least 2–3 h (unpublished data). The infested tips were replaced twice a week and checked for signs of 1st oviposition. Tips with eggs were then removed and kept in an incubator to monitor for hatch or nonviability and observations on adult activity continued until 1st hatch was obtained. Males were left with females until 1st hatch to ensure that the observed mating was successful. Mature males that had been observed previously to mate successfully were also individually confined with 6 newly emerged virgin females with observations made in the same way. Likewise, 8 newly emerged males were confined individually with mature virgin females aged 31–36 d and monitored for mating as above. The latter procedure using mature yet virgin adults provided a check against one of either sex maturing before the other.

In this study, the active oviposition period was defined as the period (in weeks) of continuous oviposition when a minimum of 2 eggs were laid per week per female. Because adelgid eggs are normally not available until the spring, the development rates of fall and winter collected adelgids were manipulated in the laboratory to provide a constant supply of adelgid nymphs, adults, and egg masses essential for stimulus of oviposition. A 2nd experiment was conducted at 25°C and a photoperiod of 16:8 (L:D) h. Thirty-four pairs of newly emerged adults were maintained as above. Preliminary monitoring had indicated that females did not start oviposition before they were 10 d old. Thereafter, infested tips were replaced daily and examined for the presence of eggs. Eggs of each pair were then held in batches at 25°C and checked for viability until hatching of the earliest batch of eggs per female was noted.

Lifetime fecundity was defined as the total number of eggs produced per female from eclosion to death. For the 1st measurement of lifetime fecundity, newly emerged adults of *P. tsugae* were sexed, paired, and kept as described above. Tips from the previous week were examined carefully under a dissecting microscope to record the number and location of viable eggs per female per week during her lifetime. Nonviable eggs were usually distinguishable by lack of turgor and the absence of the opalescent sheath surrounding the egg. Such eggs usually disintegrated on touch or a day or 2 later. Individuals (n = 19) were followed until they died. In a 2nd treatment, fresh adelgid-infested hemlock tips were provided twice a week during peak oviposition to increase the number of oviposition sites under 24 h light (n = 12) and the mean lifetime fecundity, longevity, and length of female (mm) recorded. Longevity of adults from the colony, measured in days, was also recorded for males (n = 80) and females (n = 90) maintained in the same way and only adults which lived for >2 wk were included in the calculation of means. The sex ratio was estimated by microscopic examination of 5,440 adult males reared from 13 cohorts developing on *A. tsugae* under controlled temperature conditions of 22°C and a photoperiod of 16:8 (L:D) h from January to May 1997.

**Statistical Methods.** Means were reported with the standard deviations and range for each sample and comparisons made using t-tests. The 2-sample t-test for normal data with equal variances was used to test for differences in longevity between the sexes, mean number of eggs per week and longevity of females under different experimental conditions. The Aspin-Welch unequal variance t-test for normal data with unequal variances was used for comparing male maturity estimates, lifetime fecundity, and length of the active oviposition period under different conditions. The Mann–Whitney U test for non-normal data with equal variance was used for comparing the mean maximum number of eggs per week under different experimental conditions. The Spearman rank correlation tested for relationships between lifetime fecundity and female length; and lifetime fecundity with longevity. Nonlinear regression was performed using the
Levenberg Marquardt nonlinear least-squares algorithm (Nash 1987) to establish a relationship between fecundity and female longevity. Linear regressions were also performed to investigate the relationship between fecundity and oviposition period, and the effect of female size on fecundity. The analysis of variance (ANOVA) was used to test the significance of regressions. All statistical tests were performed using NCSS 6.0 System for Windows (Hintze 1995).

Results and Discussion

Developmental Stages. The egg of *P. tsugae* is oval and reddish-orange in color within a colorless sheath, averaging 0.48 ± 0.03 (0.40–0.60) mm in length by 0.25 ± 0.02 (0.20–0.30) mm in width (n = 20). Eggs are usually deposited singly in concealed locales within the curled bud-scales, although 2 or more eggs may be deposited at a single site. Eggs are also laid in empty male cones or beneath the woolly secretions of the adelgid. This behavior is typical of the Coccinellidae, which deposit their eggs in batches, usually in exposed situations (Hagen 1962). All 4 stages of *P. tsugae* larvae are highly mobile and voracious. Exuviae are often found on hemlock needles or along the stem. The 1st instar is reddish-brown with a darker brown head while the 2nd instar is gray with a black head. Both 3rd and 4th instars are dark gray or reddish brown with black heads and legs. Table 1 gives development times of larval and pupal stages with head capsule widths for the different instars. Only late 4th instars begin to develop a partial woolly covering which is pronounced at the sedentary prepupal stage and at this time, no further feeding occurs. Thus, *P. tsugae* is unlike other members of the tribe Scymnini, whose larvae are conspicuously covered with a thick coating of wax (Kamiya 1966, LeSage 1991). During ecdisis to the pupal stage, the larval skin is shed to reveal the pupa. The pupa is initially a bright reddish-brown and darkens slightly just before emergence. Thus, in contrast to other members of the Scymnini (LeSage 1991, Hodek and Honek 1996), the pupa is not covered by the larval skin. The newly emerged adult *P. tsugae* is initially a light golden brown before gradually darkening to jet black within a day. Adult *P. tsugae* are black, with dorsal pubescence (see Sasaji and McClure [1997] for a detailed taxonomical description). The female measures 1.83 ± 0.14 (1.50–2.25) mm in length and 1.14 ± 0.08 (0.95–1.35) mm in width (n = 70), whereas the male measures 1.74 ± 0.14 (1.50–2.35) mm by 1.10 ± 0.09 (0.95–1.35) mm (n = 61).

Incubation and Larval Development Times. Recorded incubation time (Table 2) was temperature-dependent and agreed with general incubation periods (≥8 d) for coccinellids (Balduf 1935). The total development time from egg hatch to adult did not significantly differ between the sexes or whether lar-

<table>
<thead>
<tr>
<th>Stage</th>
<th>Duration, d</th>
<th>n</th>
<th>% larval DT</th>
<th>% total DT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>10.3±1.1</td>
<td>26</td>
<td>22.6 ± 14.9</td>
<td>49.4 ± 16.5</td>
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<tr>
<td>L1</td>
<td>4.4 ± 1.1</td>
<td>27</td>
<td>22.6 ± 14.9</td>
<td>49.4 ± 16.5</td>
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<tr>
<td>L2</td>
<td>2.9 ± 1.0</td>
<td>30</td>
<td>14.9 ± 9.8</td>
<td>34.5 ± 15.8</td>
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<tr>
<td>L3</td>
<td>3.2 ± 0.8</td>
<td>23</td>
<td>16.4 ± 10.8</td>
<td>33.9 ± 16.2</td>
</tr>
<tr>
<td>L4</td>
<td>9.0 ± 2.0</td>
<td>29</td>
<td>46.1 ± 31.2</td>
<td>77.2 ± 47.8</td>
</tr>
<tr>
<td>Pupa</td>
<td>9.8 ± 0.7</td>
<td>36</td>
<td>33.2 ± 13.2</td>
<td>66.5 ± 32.3</td>
</tr>
<tr>
<td>Hatch-Adult</td>
<td>28.8 ± 2.1</td>
<td>30</td>
<td>17.9 ± 10.2</td>
<td>55.8 ± 32.3</td>
</tr>
</tbody>
</table>

The duration of each instar (mean ± SD) is also expressed as a percentage of the total larval duration (L1–L4) and as a percentage of the total development time to adult. Development at 20°C occurred on a diet of developing *A. tsugae* nymphs and at 20°C on *A. tsugae* adults and ovisacs.
væe preyed primarily on adelgid eggs (32.7 ± 3.5 d) or developing nymphs (32.4 ± 3.5 d). Total development time was almost halved with an increase of 5°C in ambient temperature (Table 2). The total development time from egg to adult was =40.1 d at 20°C and 24.6 d at 25°C.

The development time of *P. tsugae* is comparable to that experimentally determined for other Scymnini. *Scymnus (Pullus) impexus* Mulans, an introduced predator of the balsam woolly adelgid, *Adelges piceae* Ratzeburg, had a development time from hatch to adult of over 40 d at 17–20°C (Delucchi 1954), whereas *S. marginicollis* Mannerheim, a predator of *Myzus persicae* Sulzer, had a development time from egg to adult of =22 d at 20–25°C and a photoperiod of 16 h (Buntin and Tamaki 1980). The majority of larval development is spent in the 4th instar (Table 2), followed by the 1st instar, a pattern that is true of coccinellids in general when prey availability is not limiting and environmental conditions are favorable (Hodek and Honek 1996).

**Mating Behavior and Preoviposition Period.** Multiple mating (i.e., mating instances that occurred on several days) was observed in 15 of 38 pairs. This proved too confusing to correlate with the 1st instance of viable oviposition to determine age at female reproductive maturity. However, 23 pairs yielded isolated observations of mating that were subsequently followed by the oviposition of viable eggs. At room temperatures of =20°C, females matured at 31.8 ± 8.2 (17–49) d; female reproductive maturity is defined here as the age at which successful mating was possible. Newly emerged virgin females confined with mature males successfully mated at 28 d followed by deposition of viable eggs a day or 2 later. It was assumed that the age at which males matured might also be estimated by the 1st observations of mating attempts before any viable oviposition by the paired female. The mean age at which males matured did not differ from either isolated observations of 1st matings or from 1st instances of multiple matings, thus allowing pooling of the data (*t* = 1.1778; *P* > 0.05). Males matured at 30.0 ± 8.0 (13–54) d; (*n* = 38) at =20°C. This was confirmed from the replicates that paired virgin males with mature females where males matured at 28.5 ± 4.3 (22–34) d; (*n* = 8). *P. tsugae* appears to have a long adult development to reproductive maturity at fluctuating room temperatures because 1st copulation usually takes place a few days after emergence for most larger coccinellids (Hodek and Honek 1996). At 25°C, males matured faster than at room temperatures, with the 1st attempts at mating noted at 18.8 ± 2.8 (15–26) d; (*n* = 21). The female’s preoviposition period was shortened to 22.4 ± 3.0 (19–31) d; (*n* = 33) at 25°C. This is longer compared with larger coccinellids like *Coccinella septempunctata* L., which have a preoviposition period of 12 d at 25°C (Phofofo and Obrzycki 1995). The mean preoviposition period at room temperature was 35.2 ± 8.2 d with 87% of females depositing their 1st viable eggs within the 1st wk after mating was observed. The latter concurs with that of Naranjo et al. (1990) where mating in *S. frontalis* F. preceded 1st oviposition by 3–4 d at all fluctuating temperatures studied. Tawfik et al. (1973) found that *S. interruptus* Goeze from Egypt had a preoviposition period of 12.7 d at 19.9°C and 4.5 d at 23.9°C.

**Longevity, Fecundity, and Sex Ratio.** There was no difference in longevity between sexes (*d* = 0.1944; *P* > 0.05). Mean life span for females was 162.9 ± 100.7 d and for males, 125.6 ± 76.6 d with no interruption for diapause when prey was available. However, because the frequency distributions appeared trimodal when graphed in age class intervals of 20 d (Fig. 1), the data for each sex was divided between 3 age classes (0–60 d; 61–240 d; 241–380 d) to reflect this high degree of variation. The mean longevity of adults and the percentage of the total in each age class were calculated. The majority of adult longevity (60% of females and 65% of males) fell in the middle age class with females averaging a life span of 143.7 ± 40.2 (61–240) d, whereas males averaged 136.8 ± 41.2 (61–240) d. Only 16.7% of females and 28.3% of males lived <60 d, whereas 23.2% of females and 6.7% of males lived over 240 d. The mean upper end of life spans in the laboratory with no diapause was 314.2 ± 8.2 d for females and 320.6 ± 22.9 d for males. This compared favorably with known life spans of coccinellids, which is =1 yr in the field for species with a long period of inactive life, although some species can undergo a 2nd hibernation (Hodek and Honek 1996). Laboratory studies on longevity of other Scymnini indicate shorter life spans. Buntin and Tamaki (1980) reported that adult *S. marginicollis* survived for >80 d at 20–25°C. Male *S. interruptus* lived 60.9 d at 23.5°C, whereas mated females lived 86.1 d at 19.9°C and 65.1 d at 23.9°C. Even in comparison to other coccinellids, *P. tsugae* showed greater longevity: *Coleomegilla maculata* lengi Timmerlake (Tribe Coccinellini) had a mean lifespan of 82.3 d over a range of constant temperatures (19–27.5°C) (Wright and Laing 1978), whereas maximum mean longevity recorded for *Coccinella novemnotata* Herbst (Tribe Coccinellini) in the laboratory was 62 d at 21.1°C (McMullen 1967). Delucchi (1954) estimated the longevity of *S. impexus* at 12–13 mo in the field. It would appear that *P. tsugae*, although exhibiting quite variable longevity, has a longer life span than that of other coccinellids as individuals can live
nearly a year in the laboratory without undergoing a diapause. In the field, *P. tsugae* would appear to have a longevity similar to, or perhaps even longer than that of *S. impexus* as the lifespans measured in the laboratory were under general conditions of continuous feeding and reproductive activity for 75% of the year. The lifespan in the field for *P. tsugae* would have to include the long hibernation period as overwintering adults have been recovered in the field (McCleare and Cheah 1998).

Under a photoperiod of 16:8 (L:D) h and a continuous supply of developing adelgids, *P. tsugae* was able to deposit eggs throughout the fall and winter into spring. This indicates facultative diapause, which is an added advantage for continuous mass rearing for 75% of the year. Lifetime fecundity and oviposition patterns were not significantly different under the two experimental regimes (*t* = -1.6917; *P* > 0.05) (Table 3). However, females from the 2nd experimental regime lived longer than females from the 1st (*t* = 4.5006; *P* < 0.05). Mean lifetime fecundity was 279.6 ± 128.7 (64–513) eggs and mean active oviposition period was 14.1 ± 5.5 (7–30) wk. This indicates that *P. tsugae* has a high fecundity in comparison to other *Scymnus* (Kawachi 1991) that reported *S. hoffmani* Weise produced a lifetime total of 115 eggs in 49 d, whereas Delucchi (1954) estimated fecundity of *S. impexus* at 80–100 eggs in 2.5–3 mo. *S. interruptus* had a mean fecundity of 33.3 eggs over 63 d at 19.9°C and 401.7 eggs over 61.4 d at 23.9°C with a high degree of variation (Tawfik et al. 1973). Lifetime fecundity was correlated with longevity (*r* = 0.6148; *P* < 0.001) but not with the length of the female (*r* = 0.1266; *P* > 0.05). Thus, the size of the female did not necessarily influence its fecundity, as is often the case with other coccinellids.

The regression of fecundity on longevity (Fig. 2) was significant (*F* = 20.9259; *df* = 1, 29; *P* < 0.001) as was the regression of fecundity on the length of the active oviposition period (*F* = 80.7510; *df* = 1, 29; *P* < 0.001)

### Table 3. Fecundity and oviposition patterns (means ± SD; range) of *P. tsugae* under different experimental conditions in the laboratory

<table>
<thead>
<tr>
<th></th>
<th>Treatment 1</th>
<th>Treatment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lifetime fecundity</strong></td>
<td>247.8 ± 86.5a</td>
<td>333.4 ± 161a</td>
</tr>
<tr>
<td><strong>Mean active oviposition period (wk)</strong></td>
<td>13.0 ± 3.8a</td>
<td>16.0 ± 7.6a</td>
</tr>
<tr>
<td><strong>Mean max. no. of eggs/wk</strong></td>
<td>38.8 ± 8.9a</td>
<td>33.6 ± 7.7a</td>
</tr>
<tr>
<td><strong>Mean ± SEM eggs/wk</strong></td>
<td>18.4 ± 0.7a</td>
<td>20.4 ± 1.5a</td>
</tr>
<tr>
<td><strong>Mean longevity (d)</strong></td>
<td>203.1 ± 54.4a</td>
<td>296.8 ± 39.2b</td>
</tr>
</tbody>
</table>

*Treatment 1: 20°C; 16:8 (L:D) h; fresh *A. tsugae* once per week. Treatment 2: room temperature; 24:0 (L:D) h; fresh *A. tsugae* twice per week. The 2 sample *t*-test was used for data with normal distributions and equal variances (mean eggs per week and longevity). The Aspin-Welch test was used for normal distributions with unequal variances (fecundity and oviposition period). The Mann-Whitney *U*-test was used for mean maximum eggs per week with non-normal distributions and equal variances. Means followed by a different letter in each row are significantly different. SEM reported where mean of the means was used.

![Fig. 2. Relationship between longevity (d) and lifetime fecundity of *P. tsugae* in the laboratory (*y* = -20.285 + 1.232 *x*; *r*² = 0.419; *P* < 0.001) (above) and lifetime fecundity as influenced by the duration of the active oviposition period (*y* = -5.519 + 20.178 *x*; *r*² = 0.736; *P* < 0.001) (below). Fecundity was measured in terms of the total number of eggs produced in an individual's lifetime. Active oviposition period was defined as the period of continuous oviposition (weeks).](image-url)
candidate for impact in the field as a biological control agent of *A. tsugae*.

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