

# Seasonal synchrony of life cycles between the exotic predator, *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae) and its prey, the hemlock woolly adelgid *Adelges tsugae* (Homoptera: Adelgidae)

Carole A. S.-J. Cheah and Mark S. McClure

Connecticut Agricultural Experiment Station, Valley Laboratory, PO Box 248, Windsor, CT 06095, U.S.A.

- Abstract**
- 1 The seasonal synchrony between the exotic predator, *Pseudoscymnus tsugae* and its prey, the hemlock woolly adelgid, *Adelges tsugae*, was investigated in field cages and in the forest in Connecticut, U.S.A. from 1997–1999.
  - 2 In early spring, egg to adult development took 45 d at 18.7 °C, 39.7 d at 20.2 °C and 31.5 d at 22.7 °C. Earliest emerging F1 adults mated and oviposited in the same year. whereas F1 and F2 females emerging later in the summer mated and reserved most of their egg complement for the following year.
  - 3 A second generation of *P. tsugae* is possible in Connecticut but may be delayed by cool mid-spring temperatures. Individuals of three generations of *P. tsugae*, including overwintering survivors, may coexist in July and August and adults can be found year-round with *A. tsugae* in infested hemlock forests.
  - 4 A linear regression model for development from egg to adult under field temperatures gave good agreement with results from constant temperature findings. The model predicted a lower development threshold of 9.5 °C and a sum of effective temperatures of 405 day °C. Development time of *P. tsugae* is shorter relative to its prey *A. tsugae* and generation time ratios of predator to prey was 0.16–0.5, with an advantage conferred on the coccinellid.
  - 5 Overwintering ability and behaviour were determined in 1998–1999 and adults remained on infested hemlock branches throughout a mild winter, becoming reproductively active in mid-April. Peak oviposition period extended from April to July, in synchrony with peak oviposition and developing stages of two generations of *A. tsugae*.

**Keywords** *Adelges tsugae*, Coccinellidae, field development, overwintering, oviposition, *Pseudoscymnus tsugae*, synchrony, *Tsuga* spp.

## Introduction

Hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae), a destructive pest of eastern hemlock, *Tsuga canadensis* Carriere, and Carolina hemlock, *T. caroliniana* Engelman, occurs in eastern U.S.A. throughout the mid-Atlantic states into southern New England, and in western U.S.A. on western hemlock, *T. heterophylla* Sargent and mountain hemlock, *T. mertensiana* Carriere. Eastern hemlock has a wide range from Georgia and Alabama in southern U.S.A. northwards into southern Canada (Godman & Lancaster, 1990)

Correspondence: Dr Carole A. Cheah. Tel: +1 860 683 4980; fax: +1 860 683 4987.

and is also an important ornamental species. Eastern and Carolina hemlocks, important components of watershed ecosystems and wildlife refuges (Quimby, 1995), are now seriously threatened by *A. tsugae* over a greater part of their natural ranges. In eastern U.S.A., *A. tsugae* is believed to have been initially introduced from its native Asia into Virginia, U.S.A. in 1951 (T. Tigner, personal communication). Trees infested with the adelgid may typically die in four or more years (McClure, 1991), depending on other stress factors, and a small proportion may survive with minimal crowns and little hope of recovery. Naturally occurring *A. tsugae* populations in eastern U.S.A. are primarily unregulated by the lack of effective natural enemies and host susceptibility, whereas populations on the west coast are not generally not damaging, probably due to host

plant resistance (McClure, 1992; McClure & Cheah, 1999). In Japan, a combination of both host resistance and natural enemies is believed to regulate adelgid populations there (McClure, 1995).

*Adelges tsugae* is an unusual species with a highly adaptive tolerance to prolonged low temperatures. Its complex, polymorphic life cycle in North America is similar to that in its native Japan (McClure & Cheah, 1999). Two wingless generations of *A. tsugae* are completed in a year: the sistens or winter generation and the progediens or summer generation (Annand, 1928; McClure, 1989). The mostly sessile, parthenogenic, new sistens generation hatches in June and July, then aestivates during the summer as dormant settled first-instar nymphs, which resume feeding and development throughout the autumn and winter, before beginning oviposition in late winter of the following year. In eastern U.S.A., these eggs begin hatching in April and develop to adults in June, constituting a second progediens generation (McClure, 1989). Some eggs also develop into winged sexuales, which die without oviposition due to the lack of a suitable alternate spruce host in North America (McClure, 1987). Progediens adults oviposit in June and July to give rise to the next overwintering sistens generation for continuation of the cycle (McClure, 1987, 1989).

In 1992, a promising new exotic species of coccinellid, *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae; Tribe Scymnini) (Sasaji & McClure, 1997), was discovered to be a common predator on *A. tsugae* in Japan, occurring in over 30% of forest and ornamental sites sampled where average adelgid mortality of over 90% was observed (McClure, 1995). This species is currently undergoing laboratory and field evaluation as a potential biological control agent (Cheah & McClure, 1996, 1998) and releases from 1995 to 1999 in Connecticut totalled 100 000 (McClure *et al.*, 1999). In 1999, a large-scale programme of experimental *P. tsugae* field releases was initiated in 10 eastern states in U.S.A. This study examines the extent to which the life cycle of *P. tsugae* is synchronized with the bivoltine, polymorphic life cycle of *A. tsugae* in eastern North America and provides phenological data for use in pest management programmes. The primary objectives were to determine if a second field generation of adults is possible in Connecticut and to determine overwintering strategies.

## Materials and methods

### General procedures

All field cage studies on adult oviposition and larval development were carried out in Windsor, Connecticut, U.S.A., within screen cages (30 × 30 × 84 cm) placed outside in a site sheltered from direct rain. Freshly collected adelgid-infested hemlock tips were replaced weekly for adult oviposition checks and every 2–3 d for larval development experiments. Integrity of infested foliage was maintained by wrapping cut ends in moistened cotton wool or by immersion in water. Individual adult and larval replicates were placed in Fisher 237 mL ventilated polyethylene containers, and larger larval cohort development replicates were placed in 4.9 L containers. Foliage was checked weekly for eggs by examination under a dissecting microscope (× 32) and

monitored for subsequent hatch. Daily maximum and minimum field temperatures were recorded with a thermograph.

### Oviposition patterns of overwintering females

Overwintering adults of *P. tsugae* were recovered alive from infested hemlock foliage at field sites in Connecticut, U.S.A., in early to mid-May (1997–1998) and in mid-March to early April (1999). The number of F1 eggs deposited per overwintering female was recorded weekly until the female's death or the end of the summer. In 1999, experimentally overwintered 1998 F1 adults provided a unique opportunity to monitor oviposition from the start of the season for a complete record of oviposition patterns. Weekly egg counts per cage gave the total number of eggs produced per cage for the season.

### Field larval development and F1–F2 oviposition patterns

In 1997, data were gathered on individual hatch and larval development times in the field for weekly cohorts of progeny produced by overwintering females. Means generated from individual records for each cohort's development to adult were compared to development time estimates calculated from average dates of oviposition per cohort and dates for ≥ 50% of hatch, pupation and adult emergence per cohort. Every 2–3 d, the number of surviving stages of *P. tsugae* was determined to provide percentage hatch, pupation and adult emergence for the whole cohort under field temperatures for estimates of dates when ≥ 50% of each cohort had hatched, pupated and emerged. These, together with the average date for oviposition per cohort provided field estimates of the duration of incubation, larval and pupal development or total development time from egg to adult for each cohort under field temperatures. Mean maximum and minimum temperatures for the period of total development from egg to adult per cohort were calculated from daily records for estimates of the mean field temperature for that cohort's period of development.

To investigate the oviposition patterns of the new F1 generation, newly emerged pairs were monitored as before ( $n = 54$  females). Weekly individual egg counts were recorded until the end of October 1997. When mortality levels increased in mid to late summer, adults were supplemented with dilute honey water every week for 2 h during foliage replenishment. Survival rates had been previously shown to be greatly stabilized by the weekly provision of honey water during the period when adelgids were aestivating (Cheah, unpublished data). Subsequently, this supplemental drinking was validated by observations in the field and laboratory of adult feeding on exudates from adelgids and aphids on adjacent foliage (Cheah; personal observations). Each female was checked for weekly oviposition from November 1997 onwards. Individual female oviposition patterns were recorded weekly until natural death after transfer to incubator conditions of 20 °C and LD 16 : 8 h in November 1997.

In 1998 and 1999, the development time of whole F1 cohorts was collectively tracked using the second percentage method to increase sample size. This method had been previously validated by 1997 results, which showed good agreement with estimates derived from individual records. For some late cohorts in 1997–1999, limited observations of percentage hatch and percentage

adult emergence were made and for these, average larval and pupal times were combined or total development time from egg to adult estimated. Mean F1 cohort total development in the field from 1997 to 1999 at different average field temperatures was compared between the years. The rate of total development was calculated as the reciprocal of the mean duration from egg to adult (1/d).

In 1998, new F1 and F2 adults were grouped separately according to age (within 4–5 d of age;  $n = 5\text{--}25$ /cage of males and females), in 17 ventilated transparent 4.7 L ( $17 \times 23.7$  cm) Nalgene polycarbonate jars for multiple mating opportunities, and checked for weekly oviposition ( $n = 143$  females). On detection of oviposition, females were then isolated individually in transparent tubes ( $10 \times 3.7$  cm) to determine which ones were ovipositing. Total numbers of F2 and F3 eggs per female per week and the percentage of female F1 and F2 adults ovipositing in 1998 were recorded. Ovipositing females were kept separate from non-ovipositing females so that a cumulative record could be kept of the percentage of reproductively mature females throughout the summer.

In 1999, 30 pairs of the earliest emerging (12–24 June) F1 adults were isolated and oviposition patterns of groups of 10 pairs per cage were monitored to confirm previous years' trends until oviposition ceased in early August. In 1998 and 1999, F2 larvae were reared in similar containers as for F1 larvae but at much smaller densities per container ( $< 10$ ) to minimize cannibalism when available prey was scarce.

#### Field sampling for predator and prey stages

For predator stage sampling in 1998, infested foliage was visually inspected at an established release site. Stages of *P. tsugae* in the field ( $n = 10\text{--}15$ ) were located, identified and the branches tagged for subsequent weekly examination to concurrently verify the developmental stages observed in field cages. Hemlocks were also sampled with a beating sheet every month from 1996 to 1999 for stages of *P. tsugae* at other field release sites where establishment of populations of *P. tsugae* had been documented (McClure & Cheah, 1998). Weekly samples of infested hemlock from various field sites in Connecticut were also examined in late spring to mid-summer in 1998 and 1999 to document the stages of late season adelgid prey available for predator life stages in the field. Ten healthy growth tips of infested hemlock (40–50 cm) were sampled randomly from different trees and the frequencies of live adelgid stages and the absence or presence of egg masses were determined for the first 100 individuals of adelgids encountered on each tip. First-instar sistens nymphs (N1) were distinguished from later instars (N2,3,4) of the progediens generation. In mid-summer, frequencies of dead progediens adults with or without eggs were also included in counts, as the former still represented available prey.

#### Autumn adult activity and overwintering behaviour

Reproductively mature adult *P. tsugae* (six females and two males) surviving the summer of 1995 were placed on

transplanted hemlock seedlings naturally infested with *A. tsugae* and kept outside in a screen cage from 10 October–14 November 1995. The infested seedlings were replaced weekly and examined carefully with a Dazor Floating Fixture lighted magnifier ( $\times 1.5$ ) for *P. tsugae* eggs. At the end of the exposure period, the adults were transferred back to room temperatures and monitored weekly for subsequent oviposition activity to verify the fertility of those individuals used for the field experiment.

In 1998, 17 cages initially set up with F1 and F2 adult *P. tsugae* from the summer remained outside until early November. The foliage was checked weekly for signs of oviposition. Loose hemlock needles and twigs were added to a depth of 5 cm in each cage to simulate leaf litter for potential overwintering sites. The litter and foliage were carefully inspected weekly for the location of adults. On average, inspection of each cage took 5–10 min at room temperature to minimize exposure to warmer temperatures. On 3 November 1998, 10 cages of adults were transferred to an incubator at  $14 \pm 1$  °C, LD 12 : 12 h for 2 weeks after inspection of the foliage and litter. These adults were then transferred to a rearing temperature of 22 °C; LD 16 : 8 h and checked weekly for resumption of oviposition.

The remaining seven cages ( $n = 45$  adults) remained exposed to field temperatures throughout the winter into spring. These included one overwintering female recovered in 1998, four females and a male collected from a field site in November and the remainder of 1998 experimental F1 offspring. The litter and foliage were checked biweekly in November and December and then monthly from mid-January to March 1999 to minimize disturbance. Fresh infested branch tips were added to each cage in late January and March 1999 to detect movement of adults between inspections. At the end of March, the number of surviving adults per cage gave an estimate of overwintering mortality. The foliage was examined for first oviposition and replaced weekly and examined as before. Inspections were also made monthly throughout the winter at established field sites. Infested foliage was either examined visually, then marked for adult presence, or sampled with a beating sheet for *P. tsugae* presence and activity.

#### Statistical analysis

Data from 1997, 1998 and 1999 were tested for normality, equality of variance and differences within the two-sample *t*-test in NCSS (Hintze, 1995). Linear regressions were fitted using the Levenberg–Marquardt non-linear least-squares algorithm for reiteration and convergence (Nash, 1987) and tested for significance using analysis of variance (Hintze, 1995). Thermal requirements for *P. tsugae* were determined from a linear regression of the mean rate of total development at different average field temperatures. The linear model used was  $1/D = aT + b$ , where  $D$  = duration of the developmental stage,  $T$  = temperature, and  $a$  and  $b$  = regression parameters. The lower development threshold (LDT) and the sum of effective temperatures (SET in day °C) for total development were calculated where  $LDT = -b/a$ , the zero intercept of the extrapolated regression line with the  $x$ -axis, and  $SET = 1/a$  (Hodek & Honek, 1996).

## Results and discussion

### Oviposition patterns of overwintering females

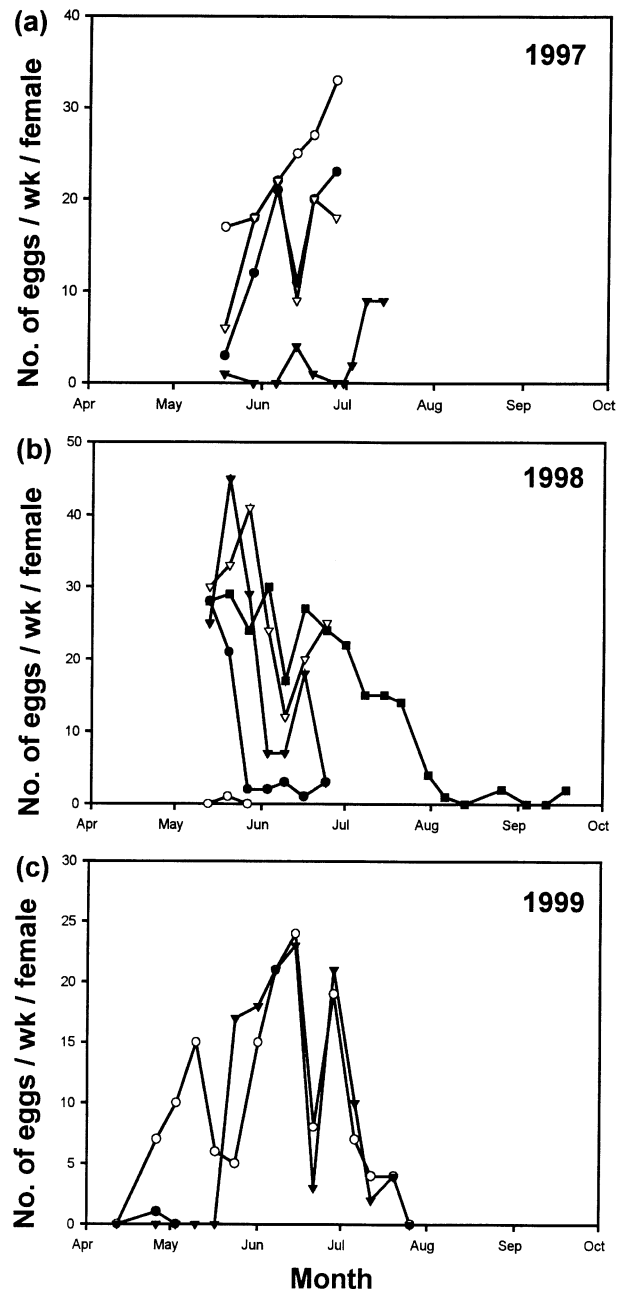
In 1997 and 1998, overwintered females recovered from release sites in mid-May oviposited immediately, indicating that oviposition had already begun in the field. In mid-March 1999, adults were recovered from the field from infested foliage at 1.8–2.5 m height on hemlocks. First oviposition of the year under external temperature conditions was noted between 13 and 26 April 1999, when the mean temperatures were  $15.2 \pm 3.0^\circ\text{C}$  (maximum) and  $3.8 \pm 2.2^\circ\text{C}$  (minimum). Similarly, oviposition in April 1999 was recorded for all caged, overwintered 1998 F1 females, together with some feeding activity. In cages with mixed populations of males and females, multiple mating was also observed in early May, although oviposition was also recorded in cages with females only.

In all three years, females readily oviposited in the absence of males, with the exception of one female recovered in 1999, which oviposited after mating. Females were generally reproductively mature and mated by the onset of winter. Individual females exhibited variable oviposition patterns (Fig. 1a–c). The mean total number of eggs deposited per female was  $87.8 \pm 48.5$  (24–142) eggs in 1997 and  $158.2 \pm 81.9$  (60–254) eggs in 1998. In 1999, field-recovered females deposited a total of 119 and 145 eggs each from April to July, whereas one female deposited only one viable egg before dying. These annual realized fecundities indicated that less than half the potential egg complement was deposited during the 1997 season, as the laboratory estimate of lifetime fecundity is  $279.6 \pm 128.7$  (64–513) eggs (Cheah & McClure, 1998). Remated females were capable of extending their oviposition period into July in 1997 and 1999. In 1998, one field-recovered female deposited 254 eggs, with sporadic low oviposition in August and September (Fig. 1b), and survived to undergo a second winter.

In 1997 and 1998, most overwintered adults died by the end of June into mid-July, whereas in 1999, 90% were dead by early August. However, four experimentally overwintered females from 1998 and 1999 survived to oviposit again in early spring 2000 after being artificially overwintered at  $9^\circ\text{C}$  from September 1999 to February 2000. Overwintering males survived until the end of June (1998) or the end of July (1997), allowing for intergenerational mating with new F1 females. Overwintering beetles, which were 1998 F1 adults with minimal or no oviposition, deposited their full egg complements in 1999 before 92% of them died by mid-August. The average lifespan measured under optimal laboratory rearing conditions of excess prey and continuous reproduction is 163 d for females and 126 d for males (Cheah & McClure, 1998) and it is evident that lifespan in the field can exceed this with overwintering survival.

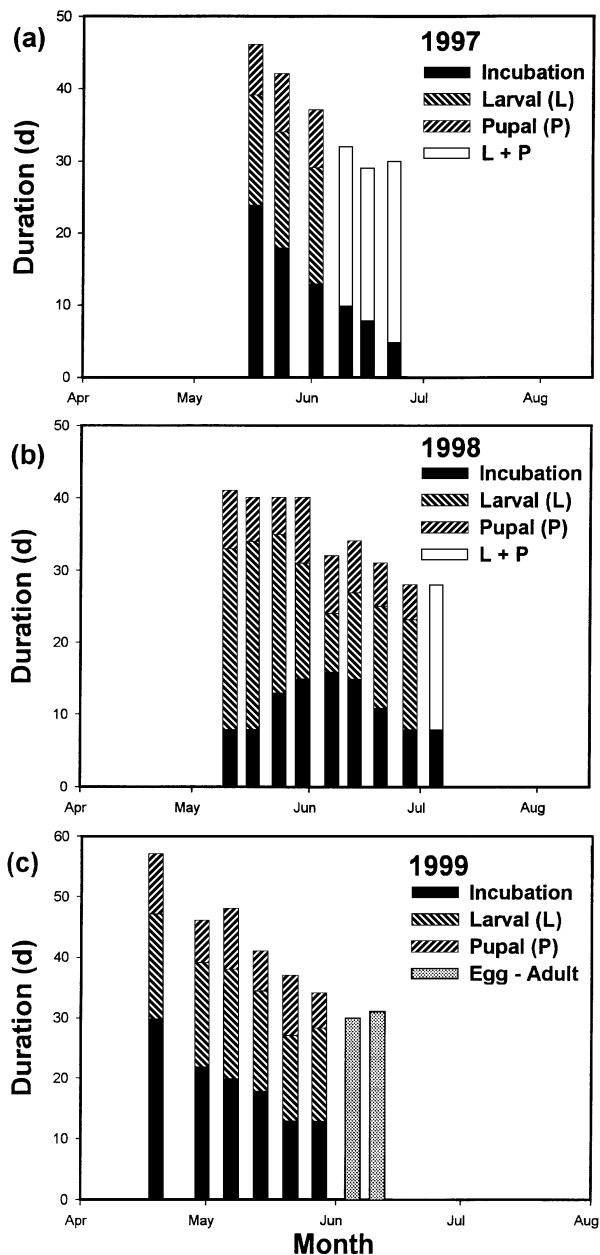
### Incubation and field development of immatures

Due to an unusually cool spring, the earliest monitored cohort of eggs deposited in mid-May 1997 did not hatch until the second week of June, together with eggs from latter May cohorts (Fig. 2a). Temperature records showed that the mean weekly daytime temperature rose to  $20^\circ\text{C}$  in the third week of May 1997, and June temperatures were  $>20^\circ\text{C}$  with minimum night



**Figure 1** Individual oviposition patterns of overwintering females recovered from field release sites in (a) 1997, (b) 1998, and (c) 1999.

temperatures  $\geq 10^\circ\text{C}$  (Fig. 3a). Development of these and subsequent batches of eggs produced F1 adults emerging between 2 and 25 July 1997. Development time from hatch to adult estimated from 50% hatch, pupation and adult emergence per cohort in 1997 correlated well with means calculated from individual data ( $r=0.9601$ ; d.f. = 4,  $P < 0.01$ ). This validated the use of the latter method for estimating total development time in the field. Development time from hatch to adult under fluctuating ambient temperatures in June and July 1997 was generally between 21 and 25 d but the key point in the predator life cycle



**Figure 2** Mean stage development times (d) for successive egg cohorts of *P. tsugae* reared at field temperatures in (a) 1997, (b) 1998, and (c) 1999. Larval (L) and pupal (P) times were combined when observations were limited.

prolonged by an abnormally cool spring was incubation time for the earliest cohorts of eggs. Incubation time decreased steadily with rising temperatures for later cohorts of eggs (Fig. 2a).

The average daily temperature in May was 13.6 °C in 1997, 17.6 °C in 1998 and 15.9 °C in 1999. This difference was reflected in much shorter incubation times for the earliest May cohorts in 1998 (Fig. 2b). However, decreasing temperatures in early June 1998 (Fig. 3b) resulted in longer incubation times for subsequent cohorts and correspondingly longer larval development for earlier cohorts. Development time ranged from 24 to 33 d for cohorts of eggs hatching in late May to mid-June. Later

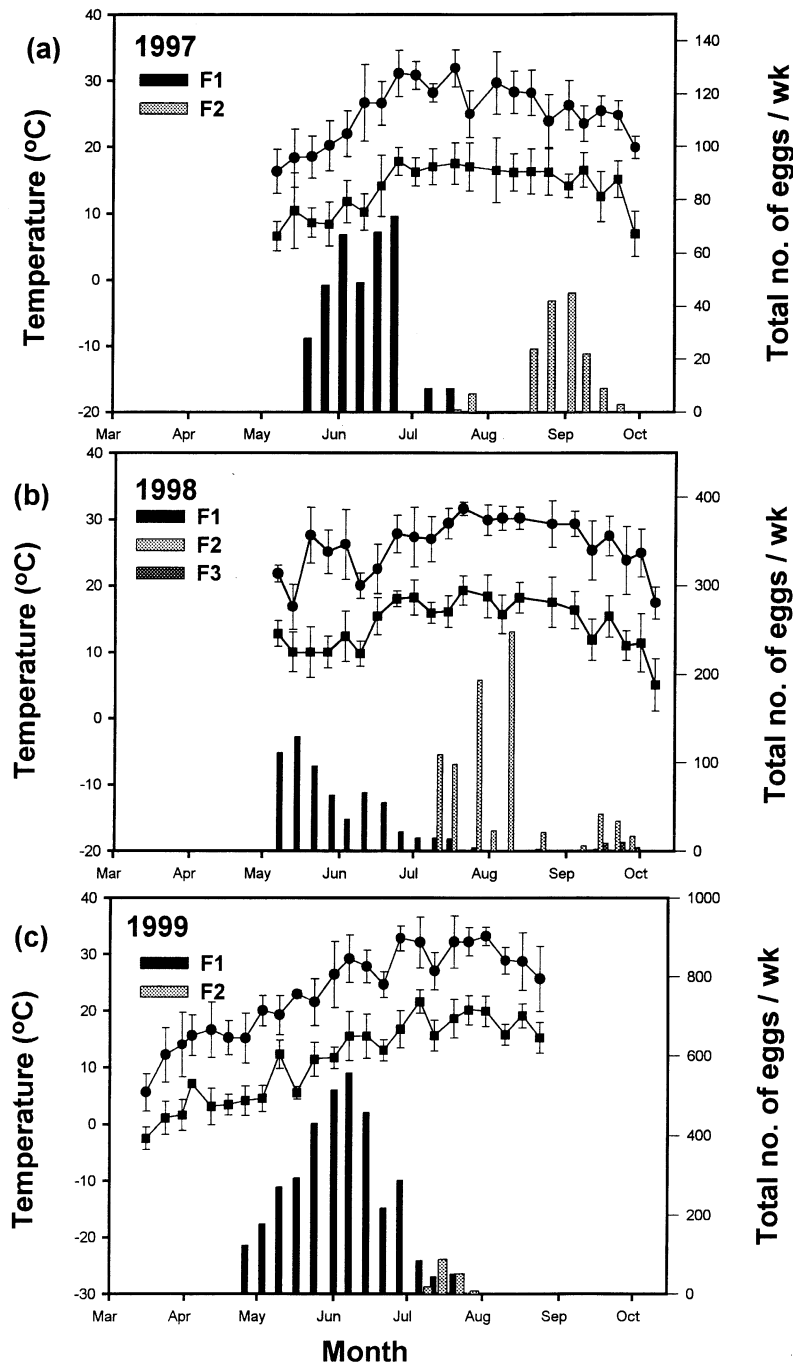
**Table 1** Stages of *P. tsugae* at forest sites in Connecticut and Virginia (1996–1999)

Year	Month of sampling	Stages present	No. of sites with <i>P. tsugae</i>	
1996	May	A *	1	
	June	A, L	1	
	July	A, L, P	2	
	August	A	1	
	September	A	2	
	October	A	2	
1997	May	A *	2	
	July	A, L, P	4	
	August	A, L	4	
	September	A, L	3	
1998	October	A	1	
	April	A *	1	
	May	A *	2	
	June	A, L, P	3 (1)	
	July	A, L	1	
	August	A, L	1	
	September	A, L	6 (2)	
	October	A	3 (1)	
	November	A	3 (1)	
	December	A	2	
	1999	January	A	1
		March	A *	1
April		A	3 (1)	
May		A, L	2 (1)	
June		A, L	1	
July		A, L	3	
August		A	4	
October		A	3	

\* denotes overwintering. A = Adult, L = Larva, P = Pupa; number in parentheses represents sites in Virginia

cohorts of eggs hatching in June and early July developed in 16–20 d with the higher summer temperatures. Mean weekly maximum temperatures in 1999 reached 22.9 °C in the second week of May (Fig. 3c). Correspondingly, the earliest cohort of eggs from mid-April had an incubation time of about a month, hatching around 19 May. Incubation time for successive cohorts decreased steadily with rising temperatures through May 1999 (Fig. 2c), a pattern similar to that in 1997. These seasonal patterns indicate that May and June temperatures have a major impact on the rate of generation turnover.

Developmental stages of larvae within field cages correlated very well with observations in the field in 1997–1999 (Table 1). Sampling in early June and July produced numerous third- and fourth-instar larvae. Fourth-instar larvae, prepupae and pupae were observed during later inspections of tagged branches in mid-June, concurrent with experimental stages recorded. Larvae were also detected in the field in August and early September 1998. Sampling at various different sites in two states over 4 years showed that *P. tsugae* remained with its adelgid prey year round. In Connecticut, at the northern end of the current adelgid distribution, adults were detected throughout the year, with larvae occurring from May to September in the field. Parallel sampling at two forest sites in Virginia, at the southern range of adelgid distribution, showed that *P. tsugae* adults were present from April to November.

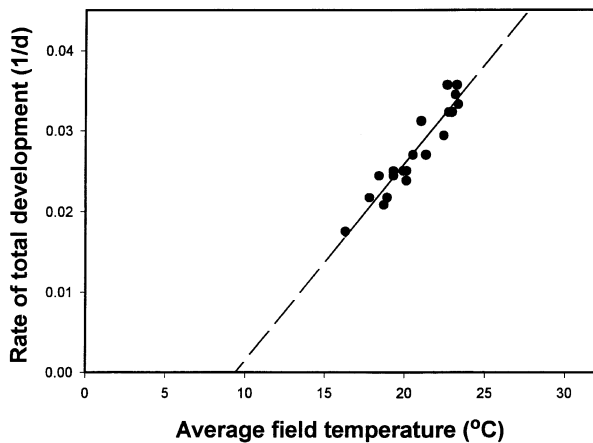


**Figure 3** Seasonal patterns of oviposition at field temperatures for overwintering, F1 and F2 generations in (a) 1997, (b) 1998, and (c) 1999. ● denotes mean maximum and ■ minimum weekly temperatures.

At average mid-spring field temperatures of 18.7 °C, *P. tsugae* took 45 d to develop from egg to adult. This is comparable to 45.3 = d at 18.7 °C for *Scymnus frontalis* (Naranjo *et al.*, 1990). Total development of *P. tsugae* took 39.7 d at 20.2 °C and was reduced to 31.5 d at 22.7 °C, for cohorts in late June to early July. *Scymnus frontalis* took 21.4 d at 26.2 °C (Naranjo *et al.*, 1990), whereas *Scymnus impexus* took 6 weeks in spring in Switzerland and Germany (Delucchi, 1954), and 31 d in Canada to develop from hatch to adult (Clark & Brown, 1961).

Estimates of the rate of total field development from egg to adult in 1997, 1998 and 1999 were compared and found not to be

different at a similar range of field temperatures ( $F=1.31$ , d.f. = 2, 18;  $P>0.05$ ). Therefore, data from all years were pooled to generate a regression model for total development rate at field temperatures. A linear regression model (Fig. 4) provided a good fit ( $y=0.00247x-0.02346$ ;  $F=175.93$ , d.f. = 1,20;  $P<0.001$ ) with a predicted LDT of 9.5 °C. SET for total development (Hodek & Honek, 1996) for *P. tsugae* predicted from the linear model is 405.1 day °C. *Scymnus frontalis* has a LDT of 11.2 °C and SET of 324 day °C (Naranjo *et al.*, 1990). By contrast, *Coleomegilla maculata lengi* (Tribe Coccinellini) has a LDT of 14.4 °C and SET of 186.6 day °C (Wright & Laing, 1978). The



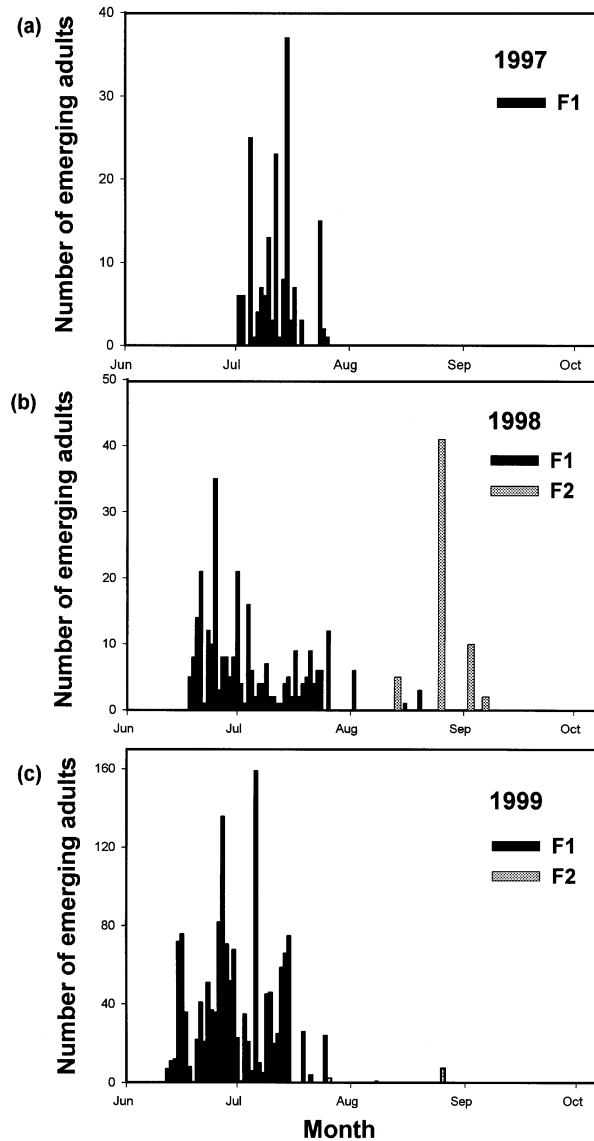
**Figure 4** The relationship between field temperatures and the rate of total development from egg to adult for *P. tsugae*. Linear regression model:  $y = 0.00247x - 0.02347$ ;  $F = 175.93$ ; d.f. = 1, 20;  $P < 0.001$ . Extrapolation to the x-axis is indicated by dashed lines.

estimates for *P. tsugae* agree with the general observation of increasing SET with decreasing LDT, which indicates a bivoltine species more adapted to extremes of temperatures in temperate zones (Hodek & Honek, 1996). 1999 was an anomalous year with mild winters, record summer temperatures and drought. *Pseudoscyrnus tsugae* survived and continued to oviposit during 8 and 16 d of daily maximums  $\geq 32^\circ\text{C}$  in June and July, respectively.

Estimates of field development from hatch to adult compared to that determined under constant temperatures indicated that there was little variation within the favourable temperature range for development. From data in Cheah & McClure (1998), total development time of 40.1 d and 24.6 d at constant temperatures of  $20^\circ\text{C}$  and  $25^\circ\text{C}$ , respectively, agreed well with values predicted from a linear regression of field development against average field temperatures (40 d and 22.2 d, respectively, from  $y = 111.37 - 3.57x$ ;  $F = 23.66$ , d.f. = 1, 20;  $P < 0.001$ ). Percentage deviation in development time under fluctuating temperatures at  $25^\circ\text{C}$  was 9.8%, which was within the 15% acceptable range (Ratte, 1985). It is concluded that this linear regression model is a useful predictor for development in the field for *P. tsugae* in Connecticut and its vicinity.

#### F1 and F2 emergence and oviposition patterns

In 1997, F1 adults emerged between 2 and 25 July (Fig. 5a). A proportion of the oldest F1 adults oviposited in July (Fig. 3a). These F2 eggs hatched but there was no initial survival to adult when reared in confined containers on aestivating adelgids. Experimental rearing conditions may have affected adelgid and larval survival in 1997. Subsequent batches of eggs deposited in August were then kept in refrigerated conditions. However, inspections and sampling of infested foliage at two established release sites in Connecticut in 1997 yielded mature larvae and young adults in mid-August and again as late as mid-September 1997, indicating the production of another generation under true field conditions. From July through September 1997, 44.4% of



**Figure 5** F1 and F2 adult emergence patterns of *P. tsugae* at field temperatures in (a) 1997, (b) 1998, and (c) 1999.

F1 females started oviposition; 79% of these were adults that had emerged earlier in the summer. In 1997, oviposition of F2 eggs was late and consequently very limited (mean number of eggs/female =  $6.4 \pm 6.0$  eggs,  $n = 24$ ). In the field, *P. tsugae* produced a partial second generation in 1997 despite the seasonal delay in development time with a cooler May and early June. In 1997, experimental F1 females reserved most of their egg complement for oviposition the following year. Under laboratory conditions, when presented with adelgids at  $20^\circ\text{C}$ , 86.5% of experimental females were ovipositing by the end of December 1997, with 96.1% ovipositing by the end of January 1998. Mean active oviposition period, defined as the period of continuous oviposition (Cheah & McClure, 1998) was  $13.6 \pm 6.6$  (3–28) weeks.

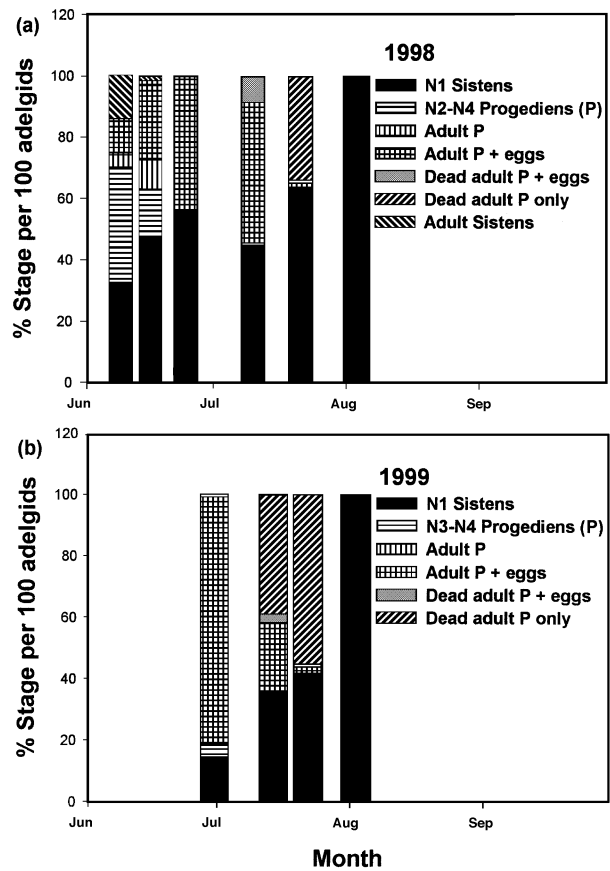
Because of a warmer start to spring oviposition and development in 1998, peak emergence of F1 adults was earlier (20 June–4 July) and adults continued to emerge throughout July and more sporadically in August (Fig. 5b). The oldest F1 females oviposited (51%; average number of eggs/female = 9.2) from 15 July to 26 August and again from 18 September to 2 October 1998 (Fig. 3b). There was minimal oviposition in September by F1 (6.3%) and F2 (16.7%) adults emerging later in the summer.

In 1997, 1998 and particularly in 1999, *P. tsugae* larvae and pupae suffered intense predation from native larvae of *Chrysoperla* sp. (Neuroptera: Chrysopidae) and nymphs of predatory Hemiptera in caged rearing conditions. The eggs or early stages of these predators were undetected during foliage replenishment of larval containers from late June to August, even with prior beating of branch tips to remove the predators. During mid to late summer, only aestivating first-instar adelgids were available and *P. tsugae* larvae and pupae fell easy prey to the larger native predators. Eggs were also attacked by native cecidomyiid larvae (Cheah, personal observations). Counts of larval and pupal cadavers of *P. tsugae* in containers where either one mature larva or adult of *Chrysoperla* sp. were present gave an estimate of predation mortality as  $17.8 \pm 15.4\%$  ( $n=8$ ). In experimental containers, native lacewing larvae also probably indirectly affected the development of *P. tsugae* by competitive depletion of progediens adelgids and egg masses in July.

Despite native predation, a second generation of adults was successfully produced in 1998, with adult emergence spanning 14 August to 7 September (Fig. 5b). From mid-August onwards, there were three coexisting generations of *P. tsugae* that contributed to the predatory impact on *A. tsugae* aestivating first instars. From 11 September to 1 October, 16.7% of F2 females started ovipositing (Fig. 3b), concurrent with minimal F1 oviposition. Fifty percent of the F3 eggs were viable and resulting larvae survived as various instars until mid-November before the majority succumbed to low temperatures. Consecutive minimum temperatures from 4 to 10 November 1998 were  $-4\text{ }^{\circ}\text{C}$  to  $-1\text{ }^{\circ}\text{C}$ . One F3 fourth-instar larva survived until the end of November. One F2 prepupa survived to the end of December through an 11 d consecutive period of minimum temperatures ( $-4.4$  to  $0\text{ }^{\circ}\text{C}$ ) and a 6 d period ( $-10$  to  $-5.6\text{ }^{\circ}\text{C}$ ) before lethal overnight minima of  $-11$  and  $-12\text{ }^{\circ}\text{C}$ . This survival demonstrated the remarkable cold tolerance of mature *P. tsugae* larvae. In 1999, a smaller replicate of F1 females emerging between 12 and 24 June oviposited from 9 to 27 July (Fig. 3c). A few F2 adults emerged in late July to late August (Fig. 5c). The successful production of the F2 generation was found to be seasonally affected by early spring temperatures in Connecticut.

#### Adelgid life stages in the field

Peak feeding activity and oviposition by the overwintering *P. tsugae* generation was synchronous with the availability of *A. tsugae* adults and eggs of the sistens generation, and developing nymphs of the second progediens generation in mid-late spring. Sampling of *A. tsugae* field stages in 1998 and 1999 showed that overlapping stages of nymphs, sistens and progediens adults with egg masses were available all through June, with progediens egg masses and hatching nymphs still present in mid-July (Fig. 6a and b). This confirms the life cycle



**Figure 6** Spring and summer stages ( $N$  = nymphal instars) of sistens and progediens *A. tsugae* generations in Connecticut in (a) 1998 and (b) 1999.

studies conducted by McClure (1987, 1989). Thus, adelgid prey is available for *P. tsugae* development and oviposition in July. F1 females oviposited F2 eggs when sexupara adults, late developing progediens adults with egg masses, and aestivating new sistens first-instar nymphs were present, although the number of eggs deposited per female on aestivating first-instar nymphs was much smaller. In late July and August, larval development was prolonged due to reduced prey quality. As adelgid survival is influenced heavily by the type of growth colonized (McClure, 1991), the provision of aestivating nymphs on new growth tips of infested hemlock ensured the healthiest adelgids available for development to adult of the F2 generation.

In late September 1998, a few live adult progediens with some viable eggs, several mobile and newly settled first- and second-instar nymphs were discovered on the new shoots of freshly collected hemlock (4.3% of 71 infested branch tips). This anomalous finding suggests that larger stages of aestivating nymphs or late adults in mid-summer may have provided the stimulus for oviposition by caged *P. tsugae* and the successful development to adult of the F2 generation in August and September 1998.



### Adult autumn activity and overwintering

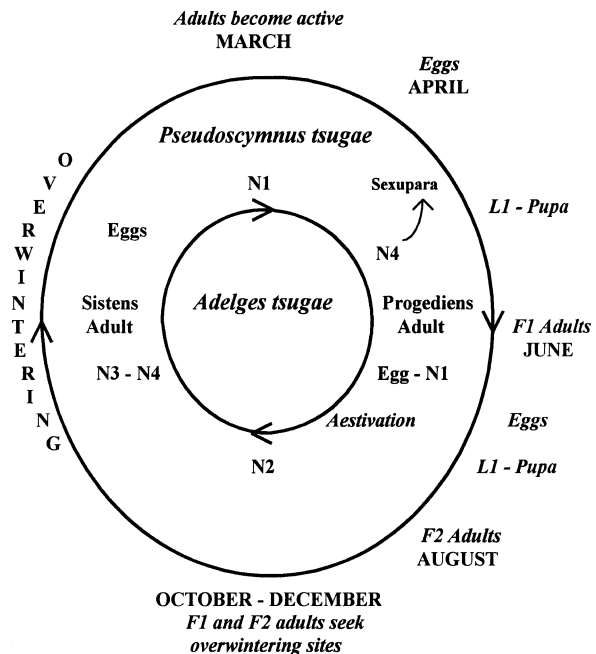
Mature adults did not oviposit when exposed to field temperatures in October and November in 1995 and 1998. Adult beetles remained on the foliage, inactive for most of the time and the majority survived minimum daily temperatures of  $-4$  to  $1^{\circ}\text{C}$  in 1995. Adults exposed to ambient autumn temperatures, when returned to laboratory conditions and given mature adelgids, were able to resume oviposition from late December 1995 to April 1996, and again in 1998–1999, confirming their reproductive potential.

In 1998, weekly inspections of the leaf litter in each cage exposed to outside temperatures revealed minimal movement in and out of the litter by 1.5–4.4% of F1 adults. No movement into the litter was recorded for the female overwintered from 1997 or adults collected in November. Adults remained singly on the foliage of caged tips, even during the lowest temperatures of the year ( $-12.2^{\circ}\text{C}$ ). Inspection with a hand lens indicated that the adults were alive but inactive. When new infested foliage was introduced into the cages in late January, movement by adults was detected in at least four cages by five adults during a February examination. *Pseudoscymnus tsugae* is capable of some movement during milder days. These observations indicate that *P. tsugae* males and females do not in general exhibit negative phototaxis nor a sustained digging reflex in response to decreasing photoperiod in order to seek out overwintering sites, at least during mild winters. There was also no propensity to aggregate, a common behavioural characteristic of coccinellids, as the majority of adults were mated before overwintering.

The results from field cage monitoring were confirmed by field observations throughout the winter. Table 1 indicates that in the field, *P. tsugae* adults remained on infested hemlock trees from October 1998 to March 1999, with some movement occurring during warmer days in the winter. This concurs with the general opinion that forest coccinellids do not leave their breeding habitat to hibernate (Hodek & Honek, 1996). In the forest, adults easily survived a 16 d period in January averaging  $-10.2 \pm 3.6^{\circ}\text{C}$  ( $-16.7$  to  $-5^{\circ}\text{C}$ ), the coldest portion of the 1998–1999 winter in Connecticut. Total overwintering mortality for caged adults was 15.6% ( $n=7$ ) by the end of March and occurred in only two of six cages.

### Synchrony of predator-prey life cycles

Synchrony between the life cycles of *A. tsugae* and *P. tsugae* in the field in Connecticut is summarized from the data gathered in this study (Fig. 7). The adelgid life cycle is primarily adapted from studies by McClure (1989, 1991), confirmed by field observations. Overwintering F1 and F2 *P. tsugae* adults from 1998 remained on infested hemlock foliage in Connecticut throughout the winter of 1998–1999, considered to be one of the warmest winters, with little or no snow cover. Three generations of *P. tsugae* are capable of overwintering, as some adults survive to undergo a second winter. Feeding and ovipositing on the adult sistens and eggs of *A. tsugae* were first recorded in April. The variability of individual annual reproduction by overwintering females is related to their age and extent of oviposition the previous year. Oldest F1 females are able to mature and initiate



**Figure 7** Seasonal synchrony between life cycles of *P. tsugae* (outer ring; L = larval instar) and its prey, *A. tsugae* (inner ring; N = nymphal instar) in Connecticut.

some oviposition in the same year, whereas F1 and F2 adults emerging later in the summer mate, but do not oviposit until the following year.

The incubation period of early eggs laid by overwintering females is affected by cool spring temperatures and, in some years, development to adult in mid-spring for early cohorts of eggs may be delayed by nearly 2 weeks. Considerable overlapping of generations occurs for developmental stages of *P. tsugae* from successive cohorts of eggs. At any one time from May to early July, all stages of the predator and prey may be encountered in the field. Adults have the remarkable ability to survive and remain dormant on infested hemlock foliage with some winter activity during intermittent periods of warmer temperatures. *Pseudoscymnus tsugae* presence at forested release sites has now been documented throughout the year, in conjunction with its adelgid prey (Table 1).

The field production of a second generation in Connecticut is significant as there are two to three overlapping generations of adult *P. tsugae* (overwintering, F1 and F2), with two generations of larvae present for a sustained predatory impact on all stages of the adelgid from mid-spring onwards until hibernation. Voltinism amongst the Scymnini is variable. Multivoltinism has been recorded for *Scymnus nubes* in California (Davidson, 1923), *S. quadrillum* in North India (Kapur, 1942), *S. interruptus* in Egypt (Tawfik *et al.* 1973), *S. cervicalis* in Pennsylvania (Wheeler & Jubb, 1979) and *S. frontalis* from Turkey (Naranjo *et al.*, 1990). In contrast, *S. impexus* is univoltine in both Europe (Delucchi, 1954) and in its country of introduction, Canada (Clark & Brown, 1961). Hodek (1967) has pointed out that multivoltine coccinellids may be more adaptable to changes in ecological conditions than univoltine species and

results here indicate the wide adaptability of *P. tsugae* to extremes in high and low seasonal temperatures. In southern New England, *P. tsugae* is capable of two field generations a year. More generations may be possible in the southern range of *A. tsugae* distribution in the mid-Atlantic states of Virginia and North Carolina where the advent of favourable spring temperatures is usually a couple of weeks earlier, at least at the lower elevations.

### Potential for biological control

Our studies on *P. tsugae* reveal that several of the desired criteria for a successful biological control agent have been realised. *Pseudoscyrnus tsugae* has so far preferentially targeted the hemlock woolly adelgid as its prey, although it will feed and develop on other adelgid species (McClure & Cheah, 1998). This agrees with known food types of Scymnini, individual genera of which show extensive specialization in accepted prey species (Hodek & Honek, 1996). Research here has demonstrated bivoltine synchrony with its primary prey, *A. tsugae*, in the field, with the predator remaining with its prey throughout the year, even during the aestivation period of the adelgid and all through the winter. Adults and larvae have the ability to survive the critical summer aestivation period of *A. tsugae*. Early observations suggest adults may supplement their diet of aestivating first-instar nymphs by feeding on aphid honeydew or at extrafloral nectaries of other forest plants. This behaviour has been documented for many species of coccinellids (Pemberton & Vandenberg, 1993; Majerus, 1994), including Scymninae (Vohland, 1996). Field records show successful adult overwintering during the winters of 1995–1996 (one of the most severe on record) 1996–1997, 1997–1998, 1998–1999 (relatively mild with little snow cover) and 1999–2000 (mild overall but with a 12 d cold period in January with minimum temperatures around  $-15^{\circ}\text{C}$ ) in a variety of sites and elevations in Connecticut and Virginia (McClure & Cheah, 1998; unpublished data for 2000), indicating adaptation to rigorous winter conditions and establishment of populations in the wild.

In general, aphidophagous coccinellids have not proved to be very effective biological control agents due to the unstable populations of their prey, which are patchily distributed and highly influenced by host plant quality (Hemptinne & Dixon, 1997). By contrast, coccidophagous coccinellids have provided more examples of better regulation (Hodek & Honek, 1996), perhaps attributable to the sessile habit and, hence, availability of their prey. Invading populations of *A. tsugae*, which can build up very quickly in the first 2 years of an infestation, do not usually begin to decline sharply until the third or fourth year, when there is little or no preferred new growth produced. Thus, the prey population in this system is less ephemeral than mobile aphid populations, which can have multiple generations fluctuating in abundance. *Pseudoscyrnus tsugae* represents an interesting test of this theory, as the potential for effective prey regulation exists, with the adelgid life cycle in North America consisting of two generations of primarily sessile stages on one host plant throughout the year. The only 'bottleneck' (Kindlmann & Dixon, 1999) in prey availability anticipated for *P. tsugae* would be in mid to late summer when predominantly aestivating sistens N1 is the only stage available, but earlier in

the season, overlapping stages of *A. tsugae* are abundant and available. *Pseudoscyrnus tsugae* also overwinters in the same forest habitat as its prey and its proximity should also confer an early advantage for potential regulation.

One hypothesis that has been recently advanced examined the relative developmental time of the predator in relation to its prey (Kindlmann & Dixon, 1999). Kindlmann & Dixon predicted that predators that have a longer developmental time than their prey are unlikely to be successful biological control agents. Aphidophagous coccinellids generally have a development time that spans several generations of aphids (Kindlmann & Dixon, 1999). Results from this study indicate that temperature-regulated *P. tsugae* F1 generation time is about 5 weeks in late spring and early summer and is similar for the F2 generation in mid to late summer. By contrast, generation times for the overwintering sistens *A. tsugae* is about 32 weeks, and for the summer progediens generation, 10 weeks (McClure, 1987). Generation time ratio is defined as the ratio of predator to prey developmental times (Kindlmann & Dixon, 1999). For this predator–prey relationship, generation time ratios are between 0.16 and 0.5. In addition, successive F1 cohorts also span the second progediens generation of *A. tsugae*. Comparison of these relative development times would seem to indicate that *P. tsugae*, once established from the start of an adelgid infestation, should prove to be an effective biological control agent of *A. tsugae*. Field releases have demonstrated significant local reductions in adelgid densities due to predation and revealed that weather, particularly the impact of winter mortality on the sistens generation, and negative density-dependent feedback at high densities of adelgids, also play important major roles in determining adelgid population dynamics (McClure, 1991; McClure *et al.*, 1999). What remains to be studied is the rate at which *P. tsugae* is able to increase its populations in the field to densities that will effect a lasting reduction in damaging levels of adelgid populations on hemlock in eastern North America.

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