Pseudoscymnus tsugae for Biological Control of the Hemlock Woolly Adelgid in Suburban Settings

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Abstract

In 1999 we released *Pseudoscymnus tsugae* on 30 adelgid-infested hemlock trees in landscapes at four locations in Rhode Island in an attempt to control the hemlock woolly adelgid, *Adelges tsugae*. Small, medium, and large trees at these locations received varying numbers of predators (ranging from 0 to 500 per tree) from mid-May to early June. Beat samples for *P. tsugae* taken on July 1 found 263 of the 4,920 beetles that were released 5 to 6 weeks earlier. However, we were unable to recover any *P. tsugae* in samples taken in October, 1999 or in April or August, 2000. There was no evidence of *A. tsugae* control from these releases.

In 2000, we standardized many variables by transplanting healthy, noninfested hemlocks into spaced plantings and then infesting them with *A. tsugae* before releasing *P. tsugae* on them. Predators were released at densities of 0, 50, 100, 200, and 300 per 1.5 to 2 m trees on June 8. Samples taken on June 26 showed that predators remained on trees in densities proportional to release numbers. Releases of 50 or more *P. tsugae* per tree significantly reduced densities of *A. tsugae* for the 2000 season. However, as in 1999, we found no eggs or larvae of *P. tsugae* in any of our samples, and adult *P. tsugae* largely disappeared a few months after release. None were recovered in samples taken in 2001.

The lack of reproduction by *P. tsugae* may be due to late releases that denied the predators access to the *A. tsugae* eggs, which are important to *P. tsugae* egg production. Predation by *Harmonia axyridis* and lacewings also appears to be important in determining *P. tsugae* survival.

Keywords:

Adelges tsugae, *Pseudoscymnus tsugae*, biological control, landscape trees.

Introduction

In 1954, the hemlock woolly adelgid *Adelges tsugae* was introduced into the eastern United States near Richmond, Virginia, and its damage was soon apparent on *T. canadensis* and *T. caroliniana* (McClure 1989). Within 5 years, *A. tsugae* was recognized as an economic threat to the forest industry, park services, homeowners, and nursery growers (Paca 1993).

A classical biological control program has been developed to control *A. tsugae* in the forest setting by using a predatory beetle, *Pseudoscymnus tsugae* Sasaji and McClure, first discovered in Honshu, Japan in 1992. Cheah and McClure (1998) found that both larvae and adult beetles feed on all stages of the hemlock woolly adelgid. The predator is capable of producing continuous generations in the laboratory, indicating its excellent potential for mass rearing as a biological control agent (Cheah and McClure1998). Initial release experiments show *P. tsugae* to have promise in reducing *A. tsugae* densities (McClure et al. 1999). Beginning in 1998, a long-term forest evaluation of *P. tsugae* was initiated in ten states in the eastern United States to evaluate the ability of *P. tsugae* to establish, disperse, and suppress *A. tsugae* (McClure and Cheah 1999).

In addition to killing hemlocks in the forest, *A. tsugae* presents a serious threat to hemlocks in landscape settings. *Tsuga* species are among the most graceful and beautiful of the large evergreens (Dirr 1990), and are commonly used in landscapes where pesticides are regularly used to keep these trees alive. This research is the first to evaluate *P. tsugae* against *A. tsugae* in the home landscape. Our specific objectives are to determine the impact of *P. tsugae* on hemlock woolly adelgid on landscape-size hemlocks under uniform conditions and to determine the impact of *P. tsugae* on hemlock woolly adelgid on hemlock trees in existing ornamental plantings.

Methods and Materials

Rearing of Pseudoscymnus tsugae. On 19 February 1999, 300 *Pseudoscymnus tsugae* adults were brought back from the New Jersey Department of Agriculture Philip Alampi Beneficial Insect Laboratory and placed into the University of Rhode Island's biological control laboratory. Rearing followed the same protocol used at the New Jersey laboratory, with slight modifications to accommodate different lab conditions (DeSanto 2001). Five hundred of the *P. tsugae* produced in the 1999 rearing program were held in a refrigerator for use in rearing in 2000. *Pseudoscymnus tsugae* rearing began on February 22, 1999, and continued until June 8, 1999 with a total production of 6,346 adult beetles. For the 2000 field season, rearing began on October 25, 1999, and lasted until July 6, 2000, with a total production of 5,196 *P. tsugae* adults.

Field Evaluation 1999. Studies were conducted in 1999 through 2000 in Washington and Providence counties of Rhode Island, to determine if *P. tsugae* could survive on established eastern hemlock and control *A. tsugae* in the landscape. The locations are as follows: Rogers Williams Park, Providence (nine trees); University of Rhode Island campus, Kingston (ten trees); Scotstun and Sprague farms, Glocester (two trees); Burlingame Management Area, Charlestown (one tree). The remaining nine trees were located on private residences -- one in Slocum and eight in Kingston (Figure 1). The hemlocks were divided into three height categories: less than 3 m, 3 m to 6 m and greater than 6m.

Pre-Release Sample and P. tsugae Release 1999. From May 12, 1999, through June 6, 1999, samples were taken to determine *A. tsugae* densities prior to *P. tsugae* release. On each sample tree we labeled ten branches, distributed around the tree less than 2 m above the ground, with florescent orange plastic marking tape. Thirty centimeters of new growth was pruned off each labeled branch.

Samples were brought back to the lab where we recorded the total number of adelgids on the underside of the new growth.

Immediately after sampling the trees, we released *P. tsugae* adults on them. The smallest trees (less than 3 m) received 0, 5, 20, 50, or 100 beetles; medium trees (3 m to 6 m) received 0, 20, 50, 100, or 200 beetles; and the largest trees (more than 6 m) received 0, 50, 100, 200, or 500 beetles (DeSanto 2001). We randomly distributed *P. tsugae* throughout the lower 2 m of each tree.

Figure 1. *Pseudoscymnus tsugae* release sites in Rhode Island in 1999. Site 1 is Glocester; 2 is URI campus; 3 is Providence; and 4 is Charlestown.

Post-Release Samples 1999. Samples were taken for *A. tsugae* on July 1 to 2, 1999; October 5 to 7, 1999; and April 22 to 24, 2000, using the same method as the pre-release samples. *Pseudoscymnus tsugae* also was sampled during these visits. In these samples a 1 m^2 Bioquip® canvas cloth was placed under a randomly selected branch. The branch was struck vigorously five times with a 2 cm diameter, 1m long wooden net handle and the number of *P. tsugae* was recorded. This procedure was repeated five times on each tree. We took additional samples for *P. tsugae* adults on these trees during July 17 to 19, 2000, and during August 29 to 30, 2000.

Field Evaluation 2000. Studies were conducted in 2000 at three sites in Washington County, Rhode Island, to determine if *P. tsugae* could become established on newly transplanted eastern hemlock in the landscape, and effectively control *A. tsugae*. The sites were located on the G.H. Gardiner Farm, the C.R. Skogley Turf Farm, and Peckham Farm, all on the University of Rhode Island Kingston campus (Figures 2 and 3). These farms are comprised of turf fields surrounded by woodlands. Forty-one hemlocks, 1.5 m - 2.0 m in height were planted on these sites at the edge of the forest, at a minimal distance of 100m apart from any other hemlock. All trees were obtained at Partyka's Nursery in South Kingstown, Rhode Island, four kilometers away from the planting sites. All trees were dug and re-planted within 48 hours. The hemlocks were healthy, and were not infested with *A. tsugae* upon transplant, which was completed March 23, 2000. The hemlocks were staked, and watered to ensure their establishment.

On April 6, 2000, we began inoculating the hemlocks with *A. tsugae*, just prior to the hatch of progredien crawlers. For this purpose we collected hemlock branches infested with *A. tsugae* progredien eggs and from these cut 30 cm twigs that were densely infested. Each tree received twenty 30 cm twigs, which were affixed by two wire twist ties. Inoculated twigs were distributed equally throughout the tree. This process was repeated on April 14 using branches not previously

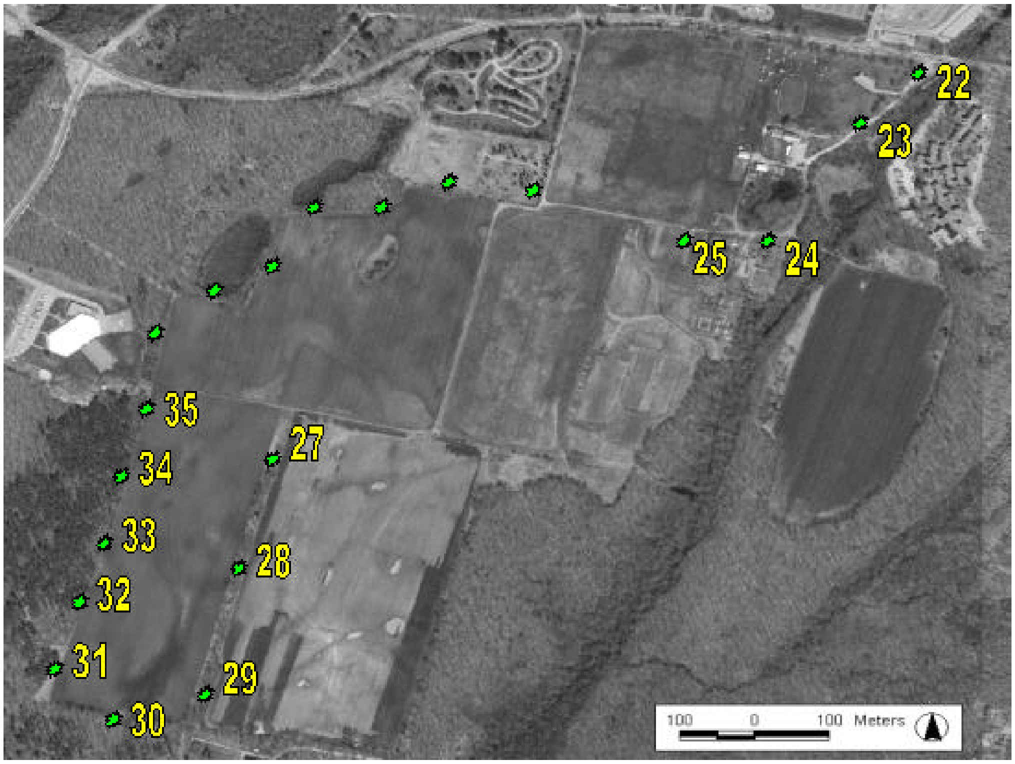

Figure 2. Locations of hemlock trees planted at the Peckham Farm at the URI Campus in Kingston, Rhode Island in 2000.

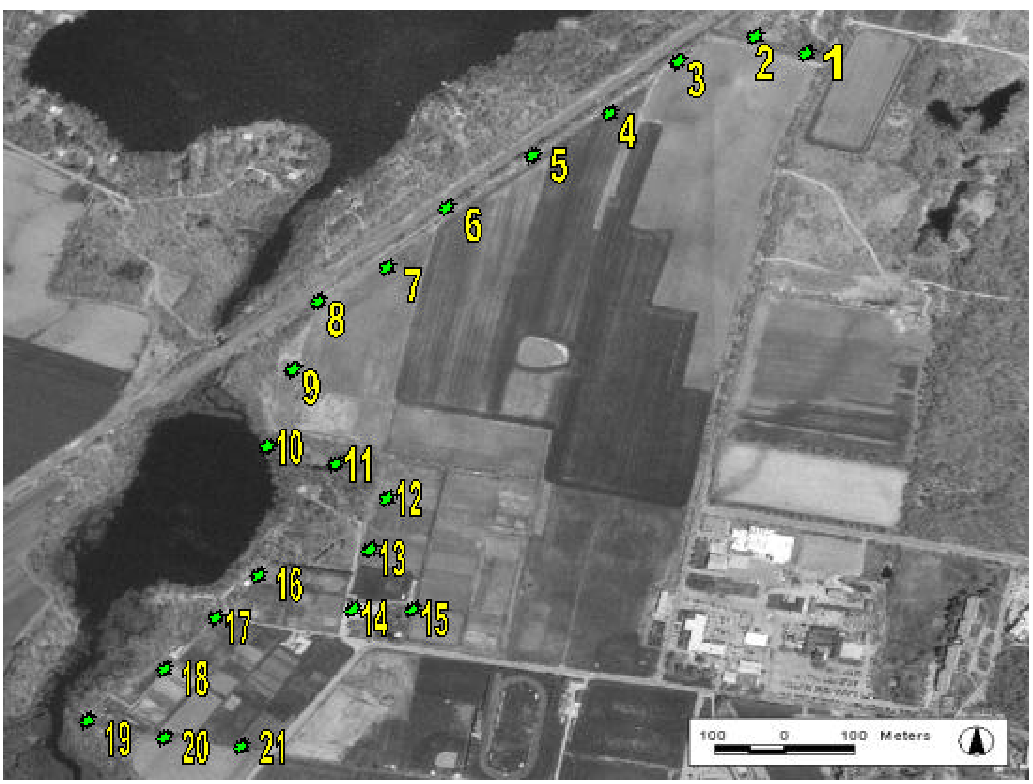

Figure 3. Locations of hemlock trees planted at the G.H. Gardiner Farm and C.R. Skogley Turf Farm at the URI Campus in Kingston, Rhode Island in 2000

inoculated. Of the 41 transplanted hemlocks, 31 were inoculated with *A. tsugae*. The trees were inspected twice a week to determine establishment of *A. tsugae*.

Pre-Release Sample 2000. On May 24, 2000, when *A. tsugae* was in the first nymphal progredien instar, ten branches were randomly selected on each tree and labeled with florescent orange plastic marking tape. With the use of 5x power binocular Opti-Visor® magnifiers, the total number of nymphs on the underside of 30 cm new growth was recorded in 10-cm increments, for each of the ten marked branches of each tree.

Pseudoscymnus Release 2000. On June 6, 2000, 3,750 adult *P. tsugae* were released on 26 trees at densities of 0, 50, 100, 200, and 300 per tree. There were five replicates of each treatment level, plus an additional release of 500 beetles on one tree. Treatments were randomly chosen for the trees and *P. tsugae* were distributed throughout the trees with a number two artists brush.

Post Release Sampling 2000. *Adelges tsugae* were sampled three times throughout the 2000 season using the same technique on the same marked branches as in the pre-release samples. On June 26, the first sample date, nymphs were in the third and fourth progredien instar and were easily counted without the use of magnifiers. Beat samples were taken for *P. tsugae* using the same procedure in 1999. Sampling for *A. tsugae* and *P. tsugae* was repeated on September 8, 2000, and November 4, 2000. First instar sistens were present on both of these sample dates. We used ANOVA to test for differences among the five treatments, followed by the Least Significant Difference test to separate means.

2001 Experiments. We performed some predation experiments in 2001 to help us understand the field results of the two previous field seasons. To determine what potential predators of *P. tsugae* were present throughout the season, we sampled four adelgid-infested trees on the URI campus with a beat stick and collecting cloth on a weekly basis throughout June and July 2001. These used the same protocol as in previous years except we used three beats per branch and we counted the predominant predatory insects present: *Harmonia axyridis* (Colepotera: Coccinellidae) and *Chrysoperla* spp. (Neuroptera: Crysopidae). On May 21, 2001 we initiated a series of laboratory tests in which a single *P. tsugae* adult or larva was placed on a piece of hemlock foliage in a small (4.5 cm) petri dish along with a single candidate predator that we collected from beat samples. Predators used in this test (Table 1) included *Harmonia axyridis, Chrysoperla* spp., and various Aranea (spiders). Treatments were checked daily and predators were removed after 5 days if prey were still alive. All exposures were repeated 10 times.

Results

Field Results 1999. Sampling on July 1, 1999, resulted in a total of 263 *P. tsugae* including six found on non-release trees at 23 and 64 m distant from release trees. No *P. tsugae* were recovered in subsequent samples taken in October 1999; April 2000; and August 2000. On the medium-size trees, there was a significant positive relationship between numbers of predators released in May and those recovered in July ($P = 0.028$, $R^2 = 0.65$), but there was no such relationship on small or large trees (DeSanto 2001).

Impact on *Adelges tsugae*. Since the samples for predators indicated that they were only present between May and July, we measured their impact on *A. tsugae* during that period. For this analysis, we compared adelgid counts on each branch, determining the density change from May 12 to July 1. When we regressed these density changes against numbers of predators released on the trees, we found no significant reductions in adelgid populations attributable to numbers of predators released in any of the three tree sizes (DeSanto 2001).

Field Results 2000. Sampling for *P. tsugae* on June 26 resulted in a total of 220 adults, recovered in numbers significantly correlated with release densities (Figure 4). The slope of this regression equation is significant at P = 2.059 x 10^{-6} and the coefficient of determination (R^2) value is 0.63. Samples taken on September 8, resulted in seven *P. tsugae* and on November 4, 2000 we found only one. The eight beetles collected in the fall of 2000 were all found on trees that had 100 beetles released on them in June. Samples taken on all trees in mid-May 2001 resulted in no *P. tsugae*. Voucher specimens from all recoveries were brought back to the lab for positive verification and then added to the voucher collection of the URI Biological Control Laboratory.

Impact on *Adelges tsugae*. The results of the branch sampling for *A. tsugae* (Figure 5) show adelgid nymphal densities increasing from May through September, and then decreasing again by November with the control densities higher than the four treatments. We evaluated the impact of *P. tsugae* by determining the change in *A. tsugae* nymphs per branch from May 24, 2000 to September 8, 2000 (Figure 6). In this analysis, all four treatments differed significantly (P = 0.05) from the control which had an average increase of 40.34 adelgids per 30 cm sample. The four

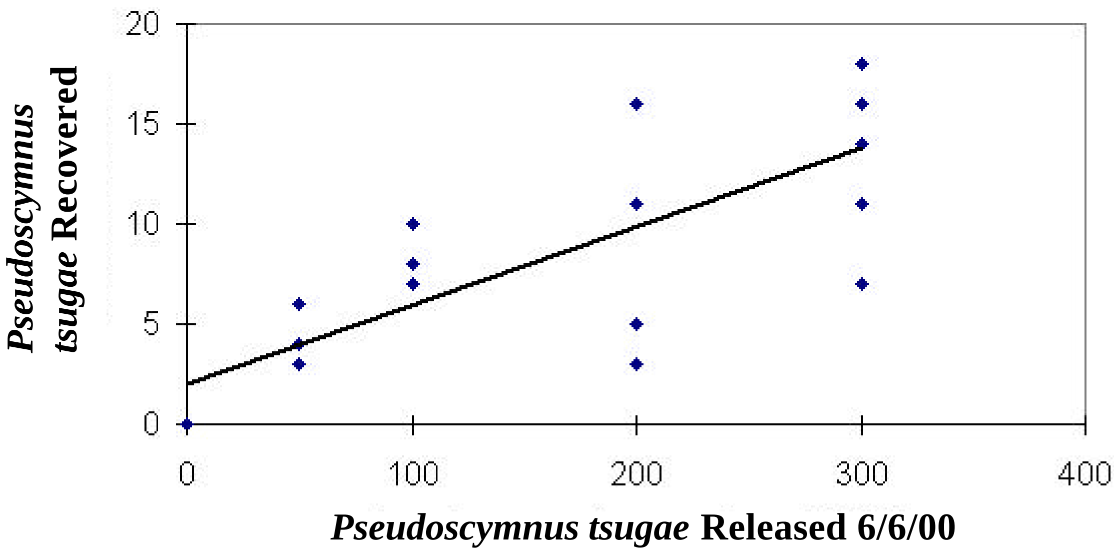

Figure 4. *Pseudoscymnus tsugae* recovered on June 26, 2000 plotted against the numbers released on the same trees on June 6, 2000. Regression line slope is significant at $P < 0.01$, R^2 = 0.63.

treatments, averaging an increase of 4.4 adelgids per 30cm sample, were not found to be significantly different from each other by ANOVA and LSD (DeSanto 2001).

2001 Experiments. The results of the predator survey on adelgid-infested hemlocks (Table 2) show that *Harmonia* adults and larvae are common in June and lacewing larvae and adults predominate in July. The petri dish study results (Table 1) show that *Harmonia axyridis* and the lacewings readily feed on immature stages of *P. tsugae* and that the lacewings also eat *P. tsugae* adults. The spiders tested also fed on *P. tsugae* larvae.

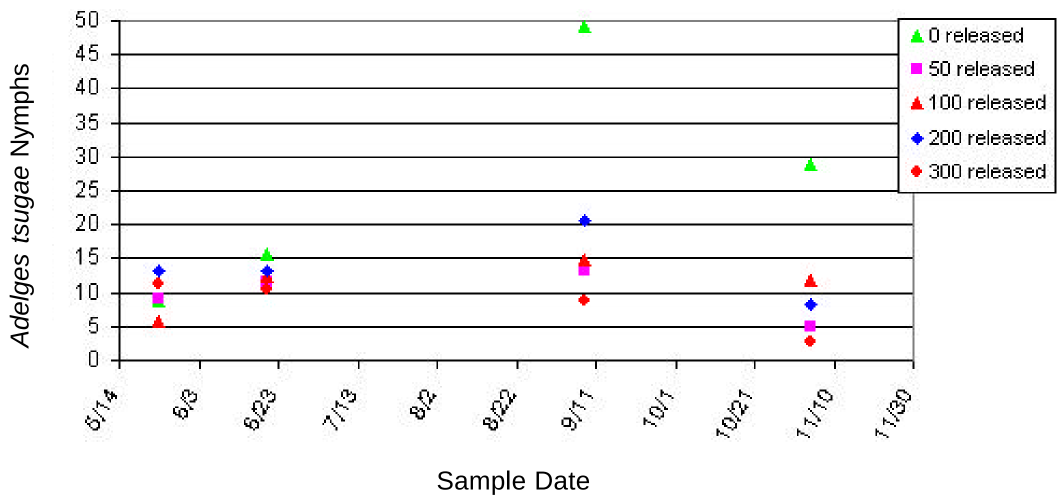

Figure 5. Mean *A. tsugae* per 30 cm branch throughout the 2000 season on trees which received different numbers of *Pseudoscymnus tsugae*.

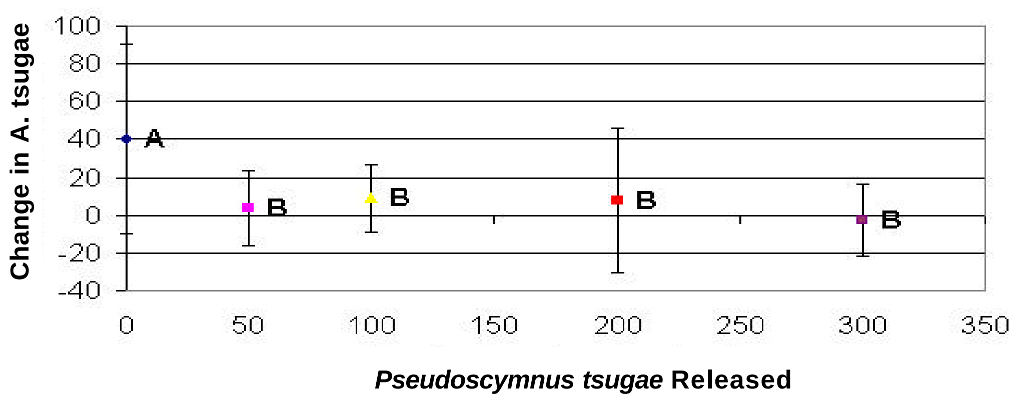

Figure 6. Mean change (+/- S.D.) in *A. tsugae* nymphal densities from May 24, 2000 (pre-release) to September 8, 2000 vs. numbers of *P. tsugae* released on trees. Change in letter denotes that the mean difference is significant at the 0.05 level.

Table 1. Results From Laboratory Experiment Testing Susceptibility of *P. tsugae* to Other Predators Collected on Hemlock Trees.[1]

Developmental Stage of *P. tsugae*	Predator	% Mortality Inflicted by Predator
eggs	*Harmonia* larva	100
	lacewing larva	100
larva	*Harmonia* larva	100
	Harmonia adult	100
	lacewing larva	100
	Aranae spp.	60
adult	*Harmonia* larva	0
	Harmonia adult	0
	lacewing larva	100
	Aranae spp.	0

[1]N = 10 replicates for all treatments.

Table 2. Results of Weekly Beat Samples of Four Adelgid-Infested Hemlocks on the URI Campus.[1]

Date	Lacewing Adults	Lacewing Larvae	*Harmonia* Adults	*Harmonia* Larvae
6/2	0	0	6	11
6/11	0	0	5	13
6/20	0	3	6	6
6/28	0	11	8	1
7/2	1	21	1	1
7/12	1	14	1	1
7/20	1	8	1	0
7/28	4	10	1	0

[1]Counts are totals from three beats on five branches on each of four trees on each date in 2001.

Discussion

1999 Field Release. As we began the 1999 season, our primary purpose was to distribute and establish *P. tsugae* throughout Rhode Island, with a secondary goal of determining how many predators are required to bring *A. tsugae* under control on individual landscape trees. This was done in a landscape setting because of the high value of these trees and their relative isolation, which facilitates experimental manipulations and sampling. Since at that time there was no information on how many predators might be needed for establishment and/or control or how far these predators would move among trees, this was very much a preliminary experiment.

The rearing and release aspects of this experiment appeared successful. The 4,920 *P. tsugae* released in 1999 were at least 28 days old, making them reproductively mature (Cheah and McClure 1998). Samples taken on July 1 indicate that many of the predators remained on the trees. On that date we found 263 beetles on release trees and only six on control trees which were 14 m and 64 m from release trees. However, we found no *P. tsugae* in any of the subsequent samples taken on these trees in October 1999, April 2000, and August 2000.

In analyzing the impact of *P. tsugae* on the adelgid populations, there was no decline in adelgid numbers on individual trees attributable to numbers of *P. tsugae* released on those trees. Instead, changes in adelgid densities appeared more related to tree condition and initial infestation level (DeSanto 2001). This observation is consistent with (M. Montgomery, personal communication) who found a significant negative correlation between the size of the parent population and the size of the next generation -- the more parents, the fewer surviving offspring. McClure (1991) also found that *A. tsugae* exhibits density-dependent feedback.

2000 Field Release. The experiments conducted in 2000 were designed to minimize the number of variables. Trees that were selected for transplanting were of uniform size, all apparently healthy with dark green foliage, and all taken from a single nursery block without any history of hemlock adelgid infestation. They were all planted 100 m apart and at least that distance from any hemlocks that were not part of the experiment. All trees inoculated with adelgids received equivalent numbers of equally infested branches.

As in 1999, the experimental setup appeared to be successful. The transplanted trees used in the experiment all remained healthy throughout the season. Laboratory rearing of *P. tsugae* was successful and 94% of the 3,750 predators that were released on June 6 were at least 28 days old and capable of ovipositing. In 2000, *P. tsugae* was recovered on 100% of the release trees in samples taken on June 26. These samples, which captured a total of 177 *P. tsugae*, indicated that the predators remained on the trees in numbers proportional to releases and none were found to move to nearby control trees. The interval between May 24 and September 8 was selected to evaluate the impact of the predators because as in 1999, predators were present during that time. (On subsequent dates in 2000, we found only eight predators and we found none in 2001.) There was time for one generation of adelgids during this sample interval. The results of this experiment indicate that releasing 50, 100, 200, and 300 *P. tsugae* per 2.5 m tree all significantly reduced *A. tsugae* population growth compared to the control treatment.

Disappearance of P. tsugae. In both years adult *P. tsugae* largely disappeared after the first post-release sample and in neither year did we recover any eggs or larvae of this predator. This may have been due to a number of factors including numbers (and quality) of predators released, timing of releases, emigration of released adults from trees, and predation on *P. tsugae*.

Cheah and McClure (2000) found that early emerging F1 generations of *P. tsugae* have the ability to mate and oviposit in the same year, whereas late-emerging F1 and F2 females mated and oviposited the following year. In rearing the predators, we began both years with a starter colony of dormant winter adults which we reared on adelgids that were primarily in the egg stage. The adult *P. tsugae* that were released in the field were mostly the F1 progeny of these adults, but these releases included many F2 progeny as well. Although the *P. tsugae* were old enough to lay eggs when released, we do not know if they did so.

Another possible problem with the releases is that the timing (early May in 1999 and early June in 2000) may have been too late in the *A. tsugae* progrediens development. Feeding upon late-instar progrediens, instead of eggs, *P. tsugae* may not have been able to produce eggs in the field (M. Montgomery, personal communication).

Predation on the *P. tsugae* eggs and larvae also may have played a role in our results. We observed large numbers of *Harmonia axyridis* larvae, lacewing larvae, and spiders in our beat samples taken in 1999 and 2000. Cheah and McClure (2000) found that lacewing larvae and predaceous hemipterans fed upon immature *P. tsugae* in laboratory studies. Our survey of predators on adelgid-infested hemlocks showed that *Harmonia* larvae were common early in June and that lacewings were more prevalent in July. Both of these species fed readily on *P. tsugae*, and the spiders that we collected on hemlocks also may be of some consequence in reducing *P. tsugae*.

We are not yet in a position to recommend releases of *P. tsugae* on landscape trees for control of hemlock woolly adelgid. Additional research might include earlier releases of *P. tsugae* and evaluation of early-season populations of potential predators of *P. tsugae* on hemlocks. This might allow released *P. tsugae* to feed on adelgid eggs, facilitating egg production of this predator. It also might allow completion of a generation before predation by *Harmonia* and lacewings begins, perhaps facilitating permanent establishment. Small, uniformly healthy trees such as we used in 2000 seem to offer good opportunities to evaluate the impact of *P. tsugae* and other predators of *A. tsugae*. In natural settings, variability in tree health, adelgid infestation levels, and proximity to other hemlocks can have a large impact on results.

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