Comparison of Numerical Response and Predation Effects of Two Coccinellid Species on Hemlock Woolly Adelgid (Homoptera: Adelgidae)

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ABSTRACT The hemlock woolly adelgid, *Adelges tsugae* Annand, is an introduced pest in North America that is native to Asia, and is causing extensive damage to eastern hemlock (*Tsuga canadensis* Carriere) and Carolina hemlock (*Tsuga caroliniana* Englemann) in the eastern United States. We compared two coccinellids imported for biological control of the adelgid: *Scymnus ningshanensis* Yu et Yao from China and *Pseudoscymnus tsugae* Sasaji and McClure from Japan. In a laboratory study, we measured the numerical response of each beetle species to a range of prey densities, and in field studies we examined the reproductive success and ability of the coccinellids to reduce populations of the hemlock woolly adelgid. In the laboratory, *S. ningshanensis* showed a positive numerical response as hemlock woolly adelgid density increased, and *P. tsugae* showed a density-independent response. In field cages, the presence of *S. ningshanensis* resulted in negative hemlock woolly adelgid population growth, in contrast to positive growth in both control cages and cages containing *P. tsugae*. Both our laboratory and field experiments suggest that *S. ningshanensis* has good potential as a biological control agent of hemlock woolly adelgid.

KEY WORDS *Adelges tsugae*, *Pseudoscymnus tsugae*, *Scymnus ningshanensis*, hemlock woolly adelgid, hemlock, biological control

THE HEMLOCK WOOLLY ADELGID (*Adelges tsugae* Annand) (Homoptera: Adelgidae) is an exotic pest in North America thought to be native to Asia. It was first reported in the United States on western hemlock, *Tsuga heterophylla* Sargent, stands in northern California and Oregon in the 1920s (Annand 1924), and it was found in Virginia in 1951 (Anonymous 1968). Hemlock woolly adelgid populations rarely occur at injurious densities on western hemlock, but damage to eastern hemlock (*Tsuga canadensis* Carriere) and Carolina hemlock (*Tsuga caroliniana* Englemann) can result in tree mortality (Orwig and Foster 1998). Native natural enemies do not adequately control *A. tsugae* in the eastern United States (Montgomery and Lyon 1996), and several potential biological control agents have been recorded in Japan and China (Sasaji and McClure 1997, Yu et al. 2000). Here we compare *Scymnus ningshanensis* Yu et Yao (Coleoptera: Coccinellidae), a coccinellid from China that is a candidate for release (Yu et al. 2000) with *Pseudoscymnus tsugae* Sasaji and McClure (Coleoptera: Coccinellidae), a coccinellid from Japan that has already been released in North America (McClure et al. 2000).

Hemlock woolly adelgid has a polymorphic life cycle that occurs on both hemlock and spruce (*Picea* spp.). There are two parthenogenic generations each year on hemlock. Adelgids from the overwintering generation are called sistens and adelgids from the spring generation are called progrediens. In New England, the eggs of the sistens hatch in July, and sistens nymphs aestivate until late fall when development resumes. Sistens become adults and lay eggs in early spring of the following year. These eggs hatch into progrediens and the nymphs develop during May and June. The progrediens have two morphs, one is wingless and remains on hemlock and the other develops to winged adults called sexuparae that fly to spruce. In North America, however, there are no species of spruce suitable for hemlock woolly adelgid, so sexual reproduction does not occur (McClure 1989). Progrediens that remain on hemlock begin oviposition in June (McClure 1989).

Scymnus ningshanensis was discovered in 1998 in Shaanxi Province, China, and was imported to the United States for evaluation before release. This species is part of a complex of at least 60 different species of natural enemies of hemlock woolly adelgid on hemlock in China (Wang et al. 1998). Coccinellids are the most abundant group of natural enemies within the complex, and most of these are in the genus *Scymnus* (Wang et al. 1998). *Scymnus ningshanensis* is univolt-

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ine and begins laying eggs in the spring after they have overwintered. Adult beetles will oviposit after feeding on all stages of *A. tsugae,* although fecundity is higher after they feed on adelgid ovisacs. An ovisac is the woolly mass that houses an adult adelgid and all of her eggs. *Scymnus ningshanensis* development time from egg to adult is ≈36 d at 20°C (Montgomery et al. 2002).

Pseudoscymnus tsugae was found in Japan on *Tsuga diversifolia* Masters and *Tsuga sieboldii* Carriere infested with hemlock woolly adelgid and on grasses and shrubs in marshes (Sasaji and McClure 1997). Adults were imported to the United States in 1994 and first released in 1995 in Connecticut (Cheah and McClure 1996). *Pseudoscymnus tsugae* is multivoltine; oviposition begins when females are 1 month old and continues for 25 wk with a mean fecundity of 300 eggs. Adults can survive on all hemlock woolly adelgid stages, but females lay more eggs after feeding on ovisacs (Cheah and McClure 1998). Development time from egg to adult is ≈40 d at 20°C (Cheah and McClure 1998). In the field, the coccinellid is multivoltine and can have two generations per year. The purpose of our study was to compare the impact of *S. ningshanensis* and *P. tsugae* on hemlock woolly adelgid from field and laboratory studies and to compare the beetles' numerical (reproductive) responses to varying densities of hemlock woolly adelgid ovisacs.

Materials and Methods

Reproductive adults were used in both experiments and were obtained from laboratory colonies. *Pseudoscymnus tsugae* adults that had eclosed 2–3 mo before testing were obtained from the Phillip Alampi Beneficial Insect Laboratory, Trenton, NJ. This is the same source of beetles used for mass releases of the coccinellid in the northeastern United States. *Scymnus ningshanensis* adults were obtained from the USDA Forest Service Insect Rearing Facility in Hamden, CT, as adult beetles that had eclosed the previous spring and overwintered at 5°C.

Laboratory Experiment to Measure Numerical Response. To determine the effect of prey density on beetle oviposition, we confined individual beetle pairs with various numbers of *A. tsugae* ovisacs. Ovisacs with sistens eggs used in the experiment were collected from the field by clipping infested hemlock twigs in late April. Twigs were 15 cm long and consisted of first and second year tree growth. We placed the twigs in wet, florist foam blocks and held them at 5°C and a photoperiod of 12:12 (L:D) h for the duration of the experiment. Infested twigs were randomly assigned to treatments, and adelgid densities on each twig were adjusted to desired levels by removing ovisacs at random.

Coccinellids were separated into mating pairs, and each pair was randomly assigned to an adelgid density and placed in a 0.5-liter paperboard cup. Each cup had a hole punched in the side through which we inserted a water pick to hold the infested hemlock twig inside the cup. Cups with beetles were held in a growth chamber at 18°C and a photoperiod of 16:8 (L:D) h. We changed the hemlock twigs weekly and counted the number of coccinellid eggs laid.

In 2000, host densities used were 0, 8, 16, 32, and 64 ovisacs per 15 cm of hemlock foliage. In 2001 densities used were 16, 32, 64, and 128 ovisacs per 15 cm of hemlock foliage. We replicated each density 10 times for each coccinellid species in each year. The experiment began 4 June 2000 and lasted 8 wk, and in 2001 the experiment began on 18 June and lasted 7 wk. The delayed start in 2001 was because of the unavailability of reproductively mature *P. tsugae* in early June.

Field Experiment. To assess the reproductive output of the two species of beetles and their impact on different densities of hemlock woolly adelgid, we confined beetles in sleeve cages placed on adelgid-infested branches on healthy hemlock trees. The sleeve cages (0.67 m × 1.0 m) were placed on 20 trees on 4 May 2001 at the Quabbin Reservoir in central Massachusetts. There were three treatments per tree: (1) no predator control bags; (2) bags with one *P. tsugae* mating pair; and (3) bags with one *S. ningshanensis* mating pair. For the experiment, we first counted the numbers of sistens ovisacs per branch. Because the number of ovisacs per branch ranged from 50 to 400, we ranked the branches by density to avoid a bias of one treatment receiving more branches with high adelgid densities. After ranking the branches, we randomly assigned one of the three treatments to each group of three adjacent densities.

We cut the bagged branches on 10 July 2001 and stored them in a cold chamber at 5°C in the laboratory until examined. We used a dissecting microscope to count adelgid and beetle progeny. Adelgids were primarily adult progrediens, but a few progrediens nymphs were also present. Beetles were all in the adult stage.

Data Analysis. Chi-square analyses were used to analyze the proportion females laying eggs at different adelgid densities. Linear regression analyses were used to analyze the numerical responses of both beetle species. The equation for the regression models was: $eggs\ laid = \beta_1(ovisac\ density) + \beta_0$. Regression models and chi-square analyses used to compare oviposition were performed using MINITAB software (Minitab 2000). A one-way analysis of variance was used to evaluate the three field treatments. Differences among treatment means were tested using Tukey's pairwise comparison at $\alpha = 0.05$. Statistical analysis of field data were performed using JMP software (SAS Institute 1995).

Results

Numerical Response in the Laboratory. In 2000, *S. ningshanensis* laid eggs for 8 wk, and *P. tsugae* laid eggs for 6 wk; in 2001, both beetle species laid eggs for 7 wk (Fig. 1). In both years, *S. ningshanensis* achieved maximum egg production in weeks 2 and 3, whereas *P. tsugae* laid the largest numbers of eggs in the first two weeks in 2000 and in the fifth week in 2001. Of those females that laid eggs over the 6–8 wk period, *S. ningshanensis* laid a mean of 61.6 ± 8.1(mean ± SE,

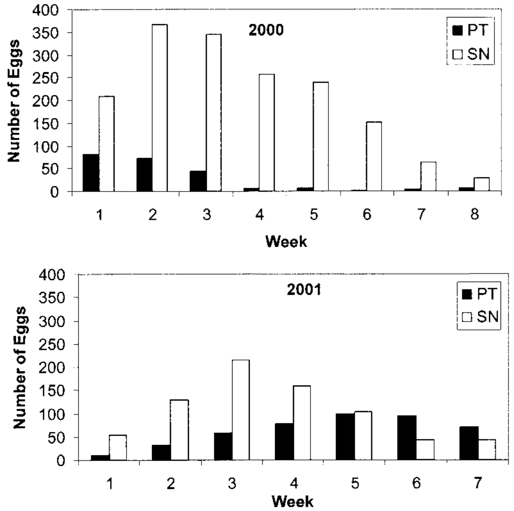

Fig. 1. The number of eggs laid each week by all female *Pseudoscymnus tsugae* (PT) and *Scymnus ningshanensis* (SN) at all densities of *Adelges tsugae.*

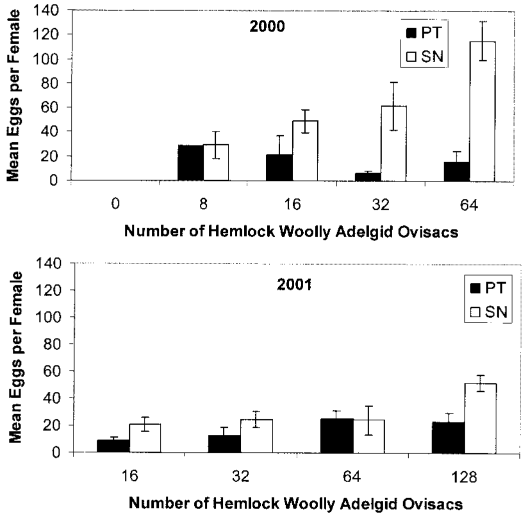

Fig. 3. Mean number eggs laid (mean ± SE) per female *Pseudoscymnus tsugae* (PT) beetle and *Scymnus ningshanensis* (SN) beetle at fixed densities of *Adelges tsugae* under laboratory conditions over 6–8 wk period.

N = 27) in 2000 and 30.4 ± 4.8 (N = 24) in 2001. In contrast *P. tsugae* laid a mean of 15.9 ± 5.5 (N = 14) in 2000 and 15.8 ± 2.6 (N = 28) in 2001.

The proportion of females that did not lay eggs in the laboratory was 50% and 30% for *P. tsugae* and *S. ningshanensis*, respectively. As *A. tsugae* ovisac densities increased (Fig. 2), the proportion *P. tsugae* females ovipositing significantly increased in 2000 (χ^2 = 9.9, df = 4, $P < 0.05$). The proportion females ovipositing decreased as *A. tsugae* ovisac densities increased in 2001, but the decrease was not significant ($P >$ 0.05). The proportion *S. ningshanensis* (Fig. 2) females that laid eggs was not significantly different at all *A. tsugae* ovisac densities above zero.

Scymnus ningshanensis fecundity increased with increasing densities of *A. tsugae* ovisacs in 2000 [($y = 1.47x + 20.2$, $R^2 = 0.46$, $P < 0.001$)] and 2001 [($y = 0.22x + 17.9$, $R^2 = 0.16$, $P < 0.01$)] (Fig. 3). *Pseudoscymnus tsugae* showed a density independent response to hemlock woolly adelgid density (Fig. 3), and the correlation between fecundity and *A. tsugae* ovisac density was not significant in 2000 [($y = -0.09x + 19.6$, $R^2 = 0.03$, $P > 0.10$)] and barely significant in 2001 [($y = 0.13x + 90.11$, $R^2 = 0.16$, $P = 0.04$)]. No females of either beetle species oviposited when zero hemlock woolly adelgid ovisacs were present.

Field Experiment. Hemlock woolly adelgid population growth (r) was computed from the equation $r = ln\ [N_t/N_0]$, where N_0 is the number sistens adults at the beginning of the experiment and N_t is the number progrediens adults and nymphs at the end. Bags in which both beetles escaped or died were

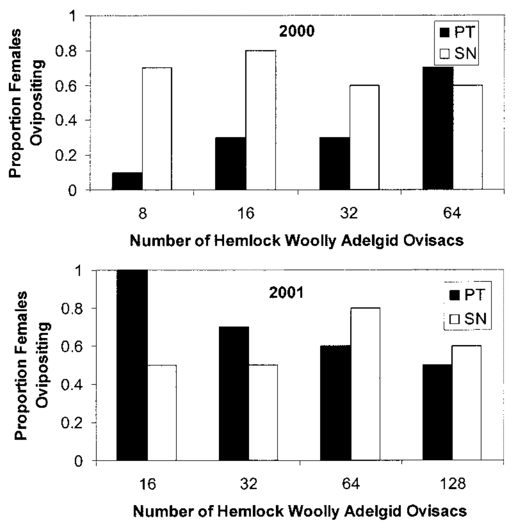

Fig. 2. Proportion *Pseudoscymnus tsugae* (PT) and *Scymnus ningshanensis* (SN) females that laid eggs at fixed densities of *Adelges tsugae* ovisacs (ovisacs/15 cm branch) in the laboratory.

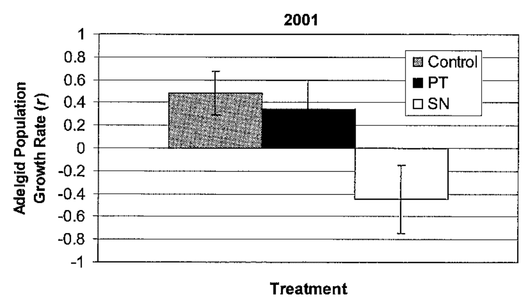

Fig. 4. The hemlock woolly adelgids' mean (±SE) population growth rates (r) in bags with no beetles (Control), bags with *Pseudoscymnus tsugae* (PT) adults, and bags with *Scymnus ningshanensis* (SN) adults.

not included in the analyses. In bags containing *S. ningshanensis,* hemlock woolly adelgid populations decreased (Fig. 4), $r = -0.45 \pm 0.30$ (SE, $N = 17$), while in bags with *P. tsugae* or in control bags (Fig. 4) adelgid populations increased: $r = 0.338 \pm 0.26$ ($N = 22$) for *P. tsugae* and $r = 0.483 \pm 0.19$ ($N = 25$) for the control.

Population growth of *A. tsugae* among all three treatments was significantly different ($F = 3.77$, $df = 63$, $P < 0.05$). Tukey's pairwise comparisons indicated that control bags and bags containing *P. tsugae* were not significantly different ($P < 0.10$), but control bags were significantly different from bags containing *S. ningshanensis* ($P < 0.05$).

In the field none of the *P. tsugae* bags contained new adults, while one-third of *S. ningshanensis* bags contained new adults. A total of 28 new *S. ningshanensis* adults were found in all bags.

Discussion

A positive numerical response is viewed as an important trait of an effective biological control agent (Huffaker 1974). Our laboratory study showed that *S. ningshanensis* could increase its egg production in response to higher hemlock woolly adelgid densities. This was not observed with *P. tsugae* that laid eggs independently of prey density. However, *P. tsugae* is multivoltine, and *S. ningshanensis* is univoltine. This fact may counterbalance the fecundity of *P. tsugae* compared with *S. ningshanensis* in our study, because *P. tsugae* can continue to lay eggs throughout the summer (Cheah and McClure 2000). Furthermore, numerical response studies conducted under laboratory conditions may not accurately predict the response under field conditions.

The fecundity and pattern of oviposition of the two coccinellids was different. *Scymnus ningshanensis* females laid more eggs than *P. tsugae* at all hemlock woolly adelgid ovisac densities. Peak oviposition for *S. ningshanensis* was during weeks two or three, whereas the peak for *P. tsugae* was week one in 2000 and week five the following year. For both coccinellids, there was a high proportion of females that did not lay eggs. The proportion *S. ningshanensis* females laying eggs was independent of prey density, and the proportion *P. tsugae* laying eggs increased with increasing prey density one year and decreased with prey density the next. Neither species laid eggs if they did not have access to adelgid eggs.

Egg production by the coccinellids in the laboratory may be influenced by the health and density of the prey. Foliage collected in 2000 was used immediately, whereas foliage collected in 2001 was stored for two weeks longer than in 2000 because the experiment was delayed as a result of the unavailability of reproductively mature *P. tsugae.* Observations of mass rearing colonies of *P. tsugae* and *S. ningshanensis* have shown that the beetles are sensitive to food quality, and egg production is highest when females have access to plentiful adelgid ovisacs from branches that are in good health (Palmer and Sheppard 2002). The specific reason for the poor level of reproduction of the beetles in our laboratory is unknown, but age and preconditioning of the beetles, food quality, and predator density may be involved. We believe factors that influence oviposition of both species should be investigated further.

The results from our field experiment complemented our laboratory study. *P. tsugae* did not significantly reduce *A. tsugae* populations when compared with the control, and no offspring were produced in the field cages. *Scymnus ningshanensis* were able to reduce hemlock woolly adelgid populations, and 20% of the females produced progeny.

Previous field experiments in which thousands of *P. tsugae* were released onto infested hemlocks indicated that *P. tsugae* had a short-term impact on *A. tsugae* population density (McClure et al. 2000). In one test, infested branches were bagged to exclude *P. tsugae* adults released on nearby, unbagged branches. Although there were lower adelgid populations on unbagged branches than on branches that were bagged to exclude the predators, the authors noted that the cages themselves may have caused an increase in adelgid survival. In another study, bagged control branches had higher numbers of hemlock woolly adelgid than unbagged control branches, presumably because the crawlers inside the bags were unable to disperse and better protected from losses because of rain and wind (M. M. and N. H., unpublished data). Other field releases (McClure et al. 2000) compared adelgid populations on branches on which 40 adult *P. tsugae* were placed, with the adelgid populations on branches on the same or more distant trees that did not receive beetles. Generally, the branches on which *P. tsugae* were placed had lower adelgid populations. We have observed (N. H. and M. M., unpublished data) that 15 adult *P. tsugae* placed in a bag on a branch with 200–300 adult adelgids will completely consume the adelgids within 3 wk. Thus, it does seem that *P. tsugae,* when present in high numbers, can have a local impact.

Overall, our field and laboratory data suggest that *P. tsugae* may be unable to produce enough progeny to adequately control high densities of hemlock woolly adelgid from one generation to the next. *Pseudoscymnus tsugae* oviposition was independent of prey density, the beetles did not reduce hemlock woolly adelgid populations, and all beetles failed to produce progeny in the field. *Scymnus ningshanensis,* however, had a positive numerical response, were able to reduce hemlock woolly adelgid populations, and some beetles did successfully produce progeny in the field. Our data show that *S. ningshanensis* is a good candidate for a biological control agent of hemlock woolly adelgid.

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