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Elizabeth E. Butin  
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**BIOLOGICAL CONTROL AND COLD-HARDINESS OF THE HEMLOCK  
WOOLLY ADELGID (HOMOPTERA: ADELGIDAE)**

A Thesis Presented

by

ELIZABETH E. BUTIN

Submitted to the Graduate School of the  
University of Massachusetts Amherst in partial fulfillment  
of the requirements for the degree of

MASTER OF SCIENCE

May 2003

Entomology

Handwritten text, possibly a signature or date, located at the top of the page.

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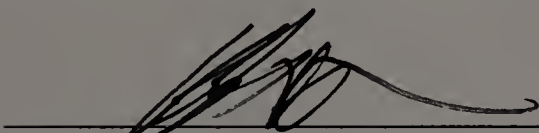
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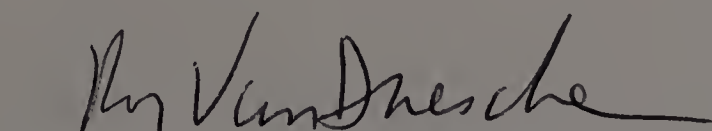
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## ABSTRACT

### BIOLOGICAL CONTROL AND COLD-HARDINESS OF THE HEMLOCK WOOLLY ADELGID (HOMOPTERA: ADELGIDAE)

MAY 2003

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Directed by: Professor Joseph S. Elkinton

The hemlock woolly adelgid, *Adelges tsugae* Annand, is an exotic pest of hemlocks in the eastern United States. Chemical and cultural controls are not feasible in a forested setting, and native natural enemies have not adequately reduced adelgid populations. My research focused on comparing the effectiveness and feeding preference of two coccinellid species (*Scymnus ningshanensis* and *Pseudoscymnus tsugae*) imported from Asia for control of the hemlock woolly adelgid. My final study tested the ability of the hemlock woolly adelgid to evolve cold-hardiness and the potential for the adelgids to invade northern New England and Canada.

To compare the effectiveness of *S. ningshanensis* and *P. tsugae*, I conducted numerical response studies in the laboratory. In the field, I measured the coccinellids' reproductive capacity and their abilities to reduce hemlock woolly adelgid populations. My laboratory studies suggest that *S. ningshanensis* has a positive density dependent numerical response, and *P. tsugae* has a density-independent response to different prey densities. In the field, *S. ningshanensis* reduced hemlock woolly adelgid populations and

produced some progeny. *Pseudoscymnus tsugae* did not reduce hemlock woolly adelgid populations and produced no progeny in the field.

To study the potential impact of *S. ningshanensis* and *P. tsugae* on non-target species, I examined the feeding preferences of both beetles along with another coccinellid often used as a biological control agent, *Harmonia axyridis*. *Scymnus ningshanensis* and *P. tsugae* adults preferred hemlock woolly adelgid, and *H. axyridis* readily consumed all prey species offered.

Finally, I investigated whether the hemlock woolly adelgid has evolved cold-hardiness to better survive lower temperatures. I found that hemlock woolly adelgids from Massachusetts are genetically different and better able to survive cooler temperatures than hemlock woolly adelgids from Maryland. It seems possible that if the hemlock woolly adelgid can adapt to even cooler temperatures, it has the potential to spread north of its current range.



# TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS .....	iv
ABSTRACT.....	v
LIST OF TABLES .....	ix
LIST OF FIGURES .....	x
CHAPTER	
1. NUMERICAL RESPONSE AND PREDATION EFFECTS OF TWO COCCINELLID SPECIES ON HEMLOCK WOOLLY ADELGID (HOMOPTERA: ADELGIDAE).....	1
Introduction.....	1
Materials and Methods.....	3
Insect Source.....	3
Numerical Response in the Laboratory.....	4
Field Experiment.....	5
Data Analysis .....	5
Results.....	6
Numerical Response in the Laboratory.....	6
Field Experiment.....	7
Discussion.....	8
Figures.....	11
2. FEEDING PREFERENCE OF THREE COCCINELLID SPECIES THAT PREDATE UPON THE HEMLOCK WOOLLY ADELGID (HOMOPTERA: ADELGIDAE) .....	15
Introduction.....	15

Materials and Methods.....	18
Source of Insects .....	18
No-choice preference tests of <i>Scymnus ningshanensis</i> .....	19
Choice Tests for Three Coccinellid Species .....	19
Data Analysis.....	21
Results.....	21
No-choice preference tests of <i>Scymnus ningshanensis</i> .....	21
Choice Tests for Three Coccinellid Species .....	21
Discussion.....	23
Tables and Figures .....	26
3. EVOLUTION OF COLD-HARDINESS IN THE HEMLOCK WOOLLY ADELGID (HOMOPTERA: ADELGIDAE).....	30
Introduction.....	30
Materials and Methods.....	33
Comparing Two Populations of <i>A. tsugae</i> .....	33
Comparing offspring of <i>A. tsugae</i> .....	34
Data Analysis .....	34
Results.....	35
Source Populations.....	35
Offspring of Source Populations.....	35
Discussion.....	37
Tables.....	40
Figures.....	41
BIBLIOGRAPHY .....	44

## LIST OF TABLES

Table	Page
2.1. Mean number prey consumed ( $\pm$ SE) in no-choice tests of <i>S. ningshanensis</i> adults feeding preference (n = 20 replicates, each with 10 prey). .....	26
2.2. Numbers of adult adelgids consumed by lady beetles during 24 given a choice between a non-target adelgid species and <i>A. tsugae</i> . .....	26
2.3. Mean time spent (minutes) in each location within the test arena during first 15 min (900 s) of access to choices. ....	27
2.4. Number of beetles found in each location after feeding for 24 h. ....	27
3.1. Results from the logistic regression of the offspring data. () = nested, * = interaction .....	40

## LIST OF FIGURES

Figure	Page
1.1. Total number of eggs laid each week by all female <i>Pseudoscymnus tsugae</i> (PT) and <i>Scymnus ningshanensis</i> (SN) at all densities of <i>Adelges tsugae</i> . .....	11
1.2. Proportion <i>Pseudoscymnus tsugae</i> (PT) and <i>Scymnus ningshanensis</i> (SN) females that laid eggs at fixed densities of <i>Adelges tsugae</i> ovisacs (ovisacs/15 cm branch) in the laboratory.....	12
1.3. Mean number eggs laid (mean $\pm$ SE) per female <i>Pseudoscymnus tsugae</i> (PT) beetle and <i>Scymnus ningshanensis</i> (SN) beetle at fixed densities of <i>Adelges tsugae</i> under laboratory conditions over 6 to 8 week period. ....	13
1.4. The hemlock woolly adelgids' mean ( $\pm$ SE) population growth rates ( $r$ ) in bags with no beetles (Control), bags with <i>Pseudoscymnus tsugae</i> (PT) adults, and bags with <i>Scymnus ningshanensis</i> (SN) adults. ....	14
2.1. Number prey consumed (mean $\pm$ SE) after 24 h in the choice test between hemlock woolly adelgid nymphs and woolly alder aphid nymphs.....	28
2.2. Mean time spent (mean $\pm$ SE) on foliage with hemlock woolly adelgid or woolly alder aphid during the first 15 min (900 s) of access to the choices.....	28
2.3. Numbers of beetles found in each location after feeding for 24 h. ....	29
3.1. Range expansion of <i>Adelges tsugae</i> in the eastern United States (Map courtesy of USDA Forest Service website) .....	41
3.2. Average percent mortality of <i>Adelges tsugae</i> collected in January 2001 from Maryland and Massachusetts and exposed to -15 °C (cold-shocked) or 2 °C (control).....	42
3.3. Average percent mortality of <i>Adelges tsugae</i> offspring reared in the same environment whose mothers were from Maryland or Massachusetts; offspring were exposed to either -15 °C (cold-shocked) or 2 °C (control).....	42

- 3.4. Average percent mortality of *Adelges tsugae* offspring reared in the same environment whose mothers were from Clarksburg, Maryland or Gaithersburg, Maryland; offspring and were exposed to either -15 °C (cold-shocked) or 2 °C (control). ..... 43
- 3.5. Average percent mortality of *Adelges tsugae* offspring reared in the same environment whose mothers were from Springfield, Massachusetts or Amherst, Massachusetts; offspring and were exposed to either -15 °C (cold-shocked) or 2 °C (control). ..... 43

## CHAPTER 1

# NUMERICAL RESPONSE AND PREDATION EFFECTS OF TWO COCCINELLID SPECIES ON HEMLOCK WOOLLY ADELGID (HOMOPTERA: ADELGIDAE)

### Introduction

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 Schaanxi 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 univoltine and begins laying eggs in the spring after they have overwintered. Adult beetles will oviposit after feeding on all stages of *A. tsugae*, but 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 approximately 36 days at 20 °C (Montgomery 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 one month old and continues for 25 weeks 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 approximately 40 days 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 impacts of *S. ningshanensis* and *P. tsugae* on hemlock woolly adelgid from field studies, and from laboratory studies compare the beetles' numerical (reproductive) responses to varying densities of hemlock woolly adelgid ovisacs.

## Materials and Methods

### **Insect Source**

Reproductive adults were used in both experiments and were obtained from laboratory colonies. *Pseudoscymnus tsugae* adults that had eclosed 2-3 months prior to testing were obtained from the Phillip Alampi Beneficial Insect Laboratory, Trenton, New Jersey. 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, Connecticut, as adult beetles that had eclosed the previous spring and overwintered at 5° C.



## Numerical Response in the Laboratory

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 eight weeks, and in 2001 began 18 June and lasted seven weeks. The delayed start in 2001 was due to 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 X 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. Since the number of ovisacs per branch ranged from 50-400, 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 Inc. 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 was performed using JMP software (SAS Institute 1995).

## Results

### **Numerical Response in the Laboratory**

In 2000, *S. ningshanensis* laid eggs for 8 weeks, and *P. tsugae* laid eggs for 6 weeks; in 2001 both beetle species laid eggs for 7 weeks (Fig. 1). In both years, *S. ningshanensis* achieved maximum egg production in weeks two and three, 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 to 8 week period, *S. ningshanensis* laid a mean of  $61.6 \pm 8.1$  (mean  $\pm$  SE,  $N = 27$ ) in 2000 and  $30.4 \pm 4.8$  ( $N = 24$ ) in 2001. In contrast *P. tsugae* laid a mean of  $15.9 \pm 5.5$  ( $N = 14$ ) in 2000 and  $15.8 \pm 2.6$  ( $N = 28$ ) in 2001.

The proportion of females that did not lay eggs in the laboratory was 50 percent and 30 percent 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 ( $\chi^2 = 9.9$ ,  $df = 4$ ,  $P < 0.05$ ). 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 [ $y = 0.13x + 9.11$ ,  $R^2 = 0.16$ ,  $P = 0.04$ ]. 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 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 to *S. ningshanensis* in our study, as *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, while 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, while foliage collected in 2001 was stored for two weeks longer than in 2000, because the experiment was delayed due to 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 pre-conditioning 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 to the control, and no offspring were produced in the field cages. *Scymnus ningshanensis* were able to reduce hemlock woolly adelgid populations, and 20 percent of the females produced progeny.

Previous field experiments where 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 due to rain and wind (M. M. and N. H. unpublished data). Other field releases (McClure et al. 2000) compared adelgid populations on branches where 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 three weeks. 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.

## Figures

Figure 1.1: Total number of eggs laid each week by all female *Pseudoscymnus tsugae* (PT) and *Scymnus ningshanensis* (SN) at all densities of *Adelges tsugae*.

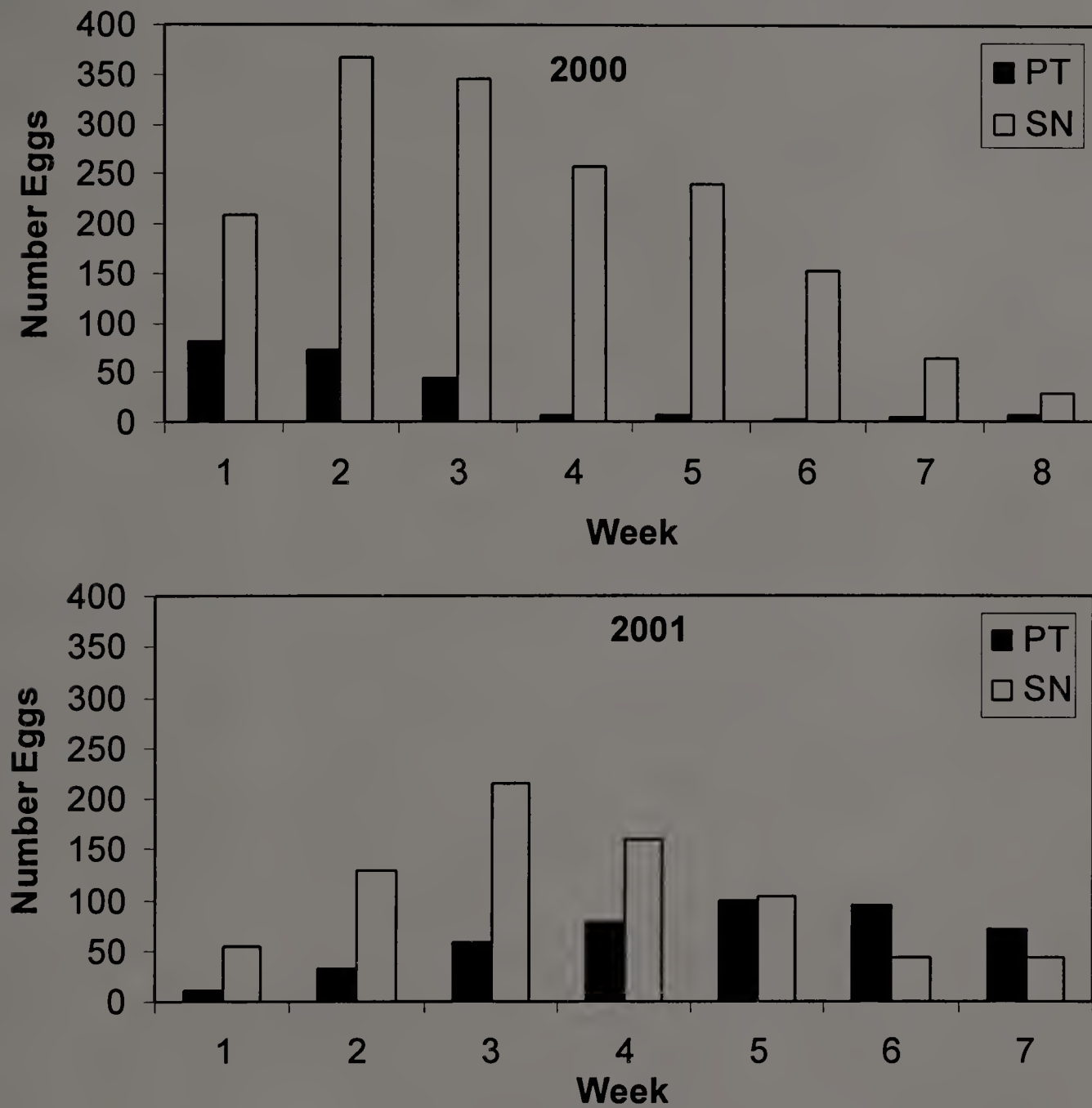




Figure 1.2: Proportion *Pseudoscymnus tsugae* (PT) and *Scymnus ningshanensis* (SN) females that laid eggs at fixed densities of *Adelges tsugae* ovisacs (ovisacs/15 cm branch) in the laboratory.

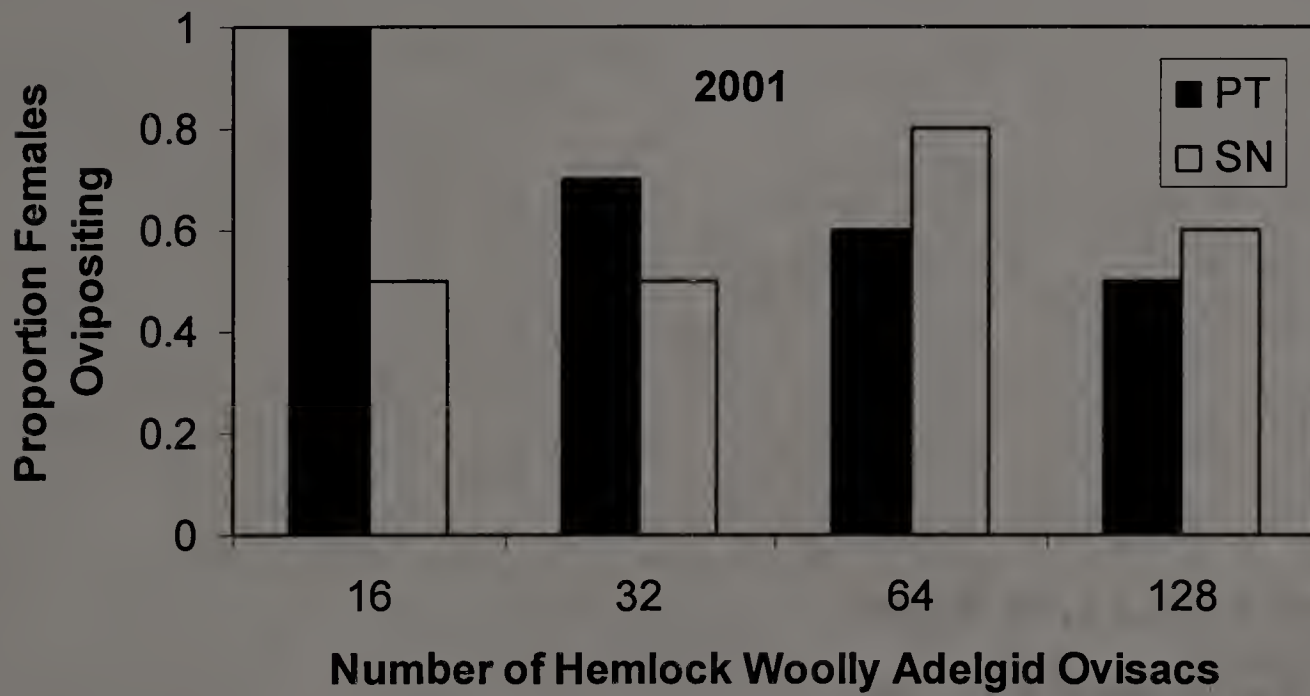
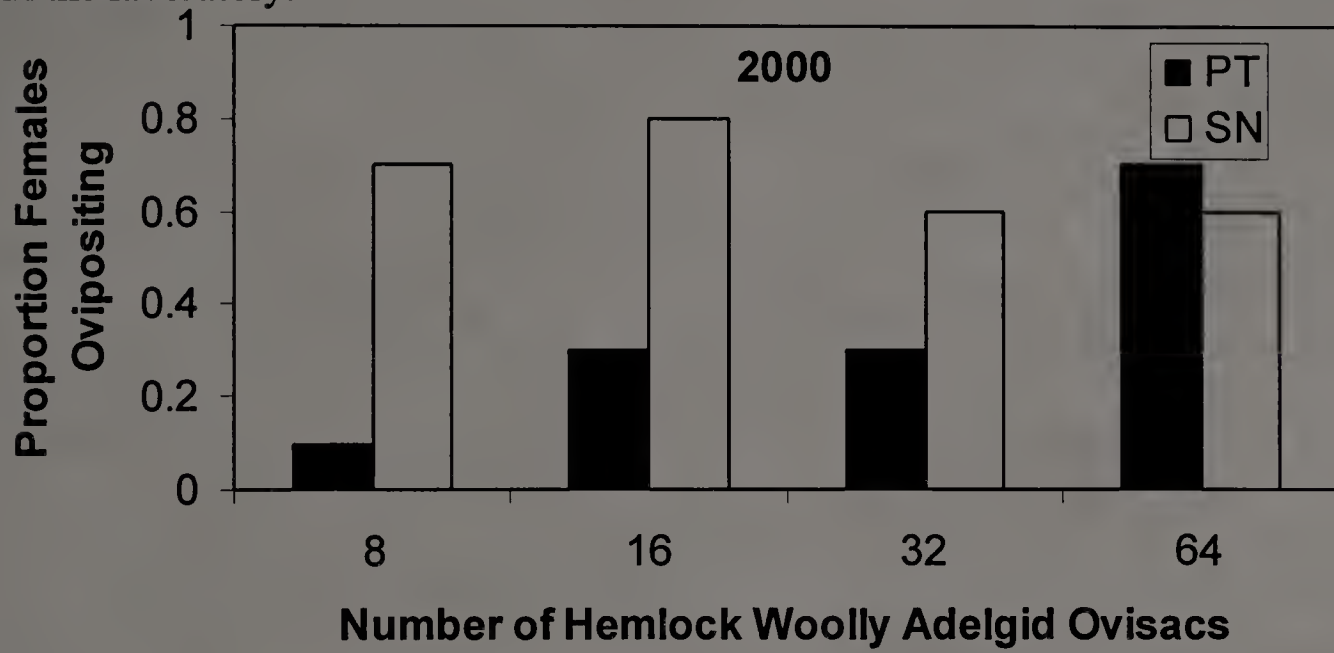


Figure 1.3: Mean number eggs laid (mean  $\pm$  SE) per female *Pseudoscymnus tsugae* (PT) beetle and *Scymnus ningshanensis* (SN) beetle at fixed densities of *Adelges tsugae* under laboratory conditions over 6 to 8 week period.

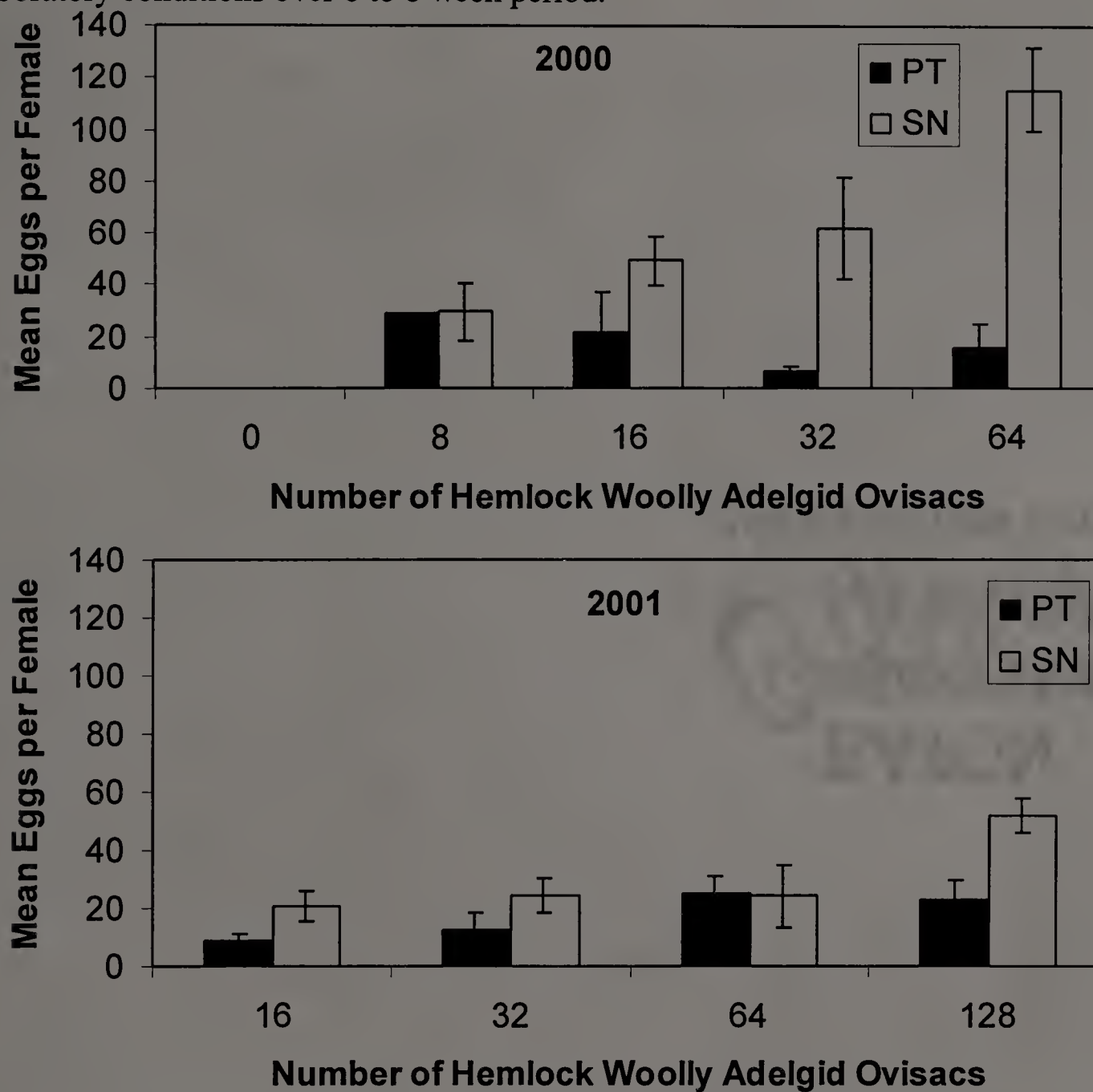
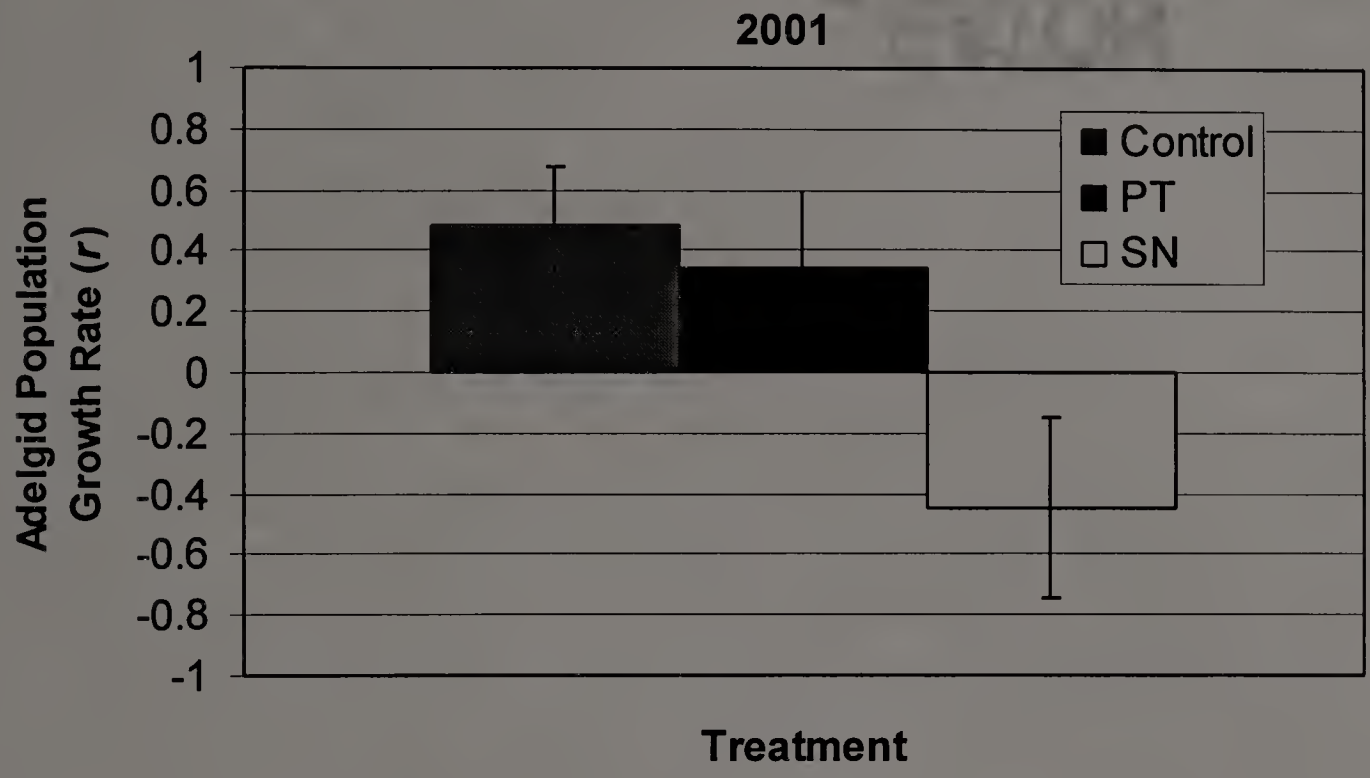


Figure 1.4: The hemlock woolly adelgids' mean ( $\pm$  SE) population growth rates ( $r$ ) in bags with no beetles (Control), bags with *Pseudoscymnus tsugae* (PT) adults, and bags with *Scymnus ningshanensis* (SN) adults.



## CHAPTER 2

# FEEDING PREFERENCE OF THREE COCCINELLID SPECIES THAT PREDATE UPON THE HEMLOCK WOOLLY ADELGID (HOMOPTERA: ADELGIDAE)

### Introduction

The hemlock woolly adelgid, *Adelges tsugae* Annand, is found in Asia and western North America wherever hemlocks occur (Blackman and Eastop 1994). It was first discovered in eastern North America in Virginia in 1951 (Anonymous 1968). Hemlock woolly adelgid now occurs in the mid-Atlantic states and southern New England on eastern hemlock, *Tsuga canadensis* Carriere, and on Carolina hemlock, *Tsuga caroliniana* Englemann (Orwig and Foster 1998). Western hemlock, *Tsuga heterophylla* Sargent, and mountain hemlock, *T. mertensiana* Carriere, on the west coast of the United States are resistant to *A. tsugae*, but hemlock species on the east coast can be severely injured or killed by *A. tsugae* (Orwig and Foster 1998).

*Adelges tsugae* is parthenogenic in the United States and has a polymorphic life cycle with two generations per year: the sistens (winter generation) and progrediens (summer generation). Sistens hatch in early summer, settle on new growth, and nymphs enter aestivation until fall, when development resumes. Adult sistens mature and lay eggs in early spring of the following year; these crawlers are called progrediens. There are two nymphal morphs of the progrediens generation, one that remains on hemlock and another that develops wings and flies to spruce (*Picea* spp). However, in North America there are no suitable spruce species for *A. tsugae* (McClure 1989).

Chemical control of the hemlock woolly adelgid is not feasible in forested areas, and native natural enemies have not suppressed *A. tsugae* populations in North America (Montgomery and Lyon 1996). The most promising control option for *A. tsugae* is classical biological control, or the importation, release, and establishment of non-native natural enemies for control of exotic pests (Cheah and McClure 1996). There are potential ecological consequences that must be considered before releasing an exotic biological control agent. One of these consequences is the interaction of the new biological control agent with non-target species, and it has been determined that some agents can have negative impacts on non-target species (Simberloff and Stiling 1996, Follett and Duan 2000, Strong and Pemberton 2000). Host preference tests can give an indication of a biological control agent's potential impact on non-target species.

The search for natural enemies of *A. tsugae* has focused on China and Japan. In both countries, the most abundant natural enemies of *A. tsugae* were coccinellids (Sasaji and McClure 1997, Wang et al. 1998). *Pseudoscymnus tsugae* Sasaji and McClure was imported from Japan in 1992, released throughout Connecticut in 1995, and is now established in localized populations throughout Connecticut (McClure et al. 2000). *Scymnus ningshanensis* Yu et Yao was imported from the Yunnan province of China in 1998, and is a candidate for release (Yu et al. 2000).

In the laboratory, *P. tsugae* is multivoltine. In contrast, *S. ningshanensis* appears to be functionally univoltine, and newly emerged adults require several weeks to reach reproductive maturity (Montgomery et al. 2002). The development time of *P. tsugae* from egg to adult is 40 d at 20 °C (Cheah and McClure 1998), and the development time of *S. ningshanensis* is 36 d at 20 °C (Montgomery et al. 2002). Adult *P. tsugae* can

survive on all life stages of hemlock woolly adelgid, but oviposit more readily after feeding on adelgid eggs (Palmer and Sheppard 2002). *Scymnus ningshanensis* adults require hemlock woolly adelgid eggs for survival and oviposition (Montgomery et al. 2002).

*Harmonia axyridis* Pallas, a native of Asia, has been released for biological control of arboreal aphids (Hagen et al. 1999), and has been shown to feed on *A. tsugae* (Wallace and Hain 2000). *Harmonia axyridis* is considered a nuisance because it invades houses (Wheeler 1995), and it has a wide host range (Hagen et al. 1999). In the laboratory *H. axyridis* has fed and developed on native lacewings (Phoofolo and Obrycki 1998), native coccinellid species (Cottrell and Yeargan 1998, Yasuda et al. 2001), and eggs of at least two species of Lepidoptera (Abdel-Salam and Abdel-Baky 2001, Ferran et al. 1997).

Environmental organizations, public officials, and land managers are concerned about potential impacts of coccinellids such as *P. tsugae*, *S. ningshanensis*, and *H. axyridis* on non-target species, especially on woolly alder aphid, *Prociphilus tessellatus* Fitch. *Prociphilus tessellatus* is the primary prey of the harvester butterfly, *Feniseca tarquinius* Fabricius, the only predaceous lepidopteran in North America. Larvae of the harvester butterfly depend upon woolly alder aphid and several other woolly species of Homoptera to complete their life cycle (Scott 1997).

It is important to test the host preferences of *P. tsugae* and *S. ningshanensis* because they have not been documented. The impacts of *P. tsugae*, *S. ningshanensis*, and *H. axyridis* on woolly alder aphid in New England have not been shown. *Pseudoscymnus tsugae* has already been mass released throughout Connecticut, Massachusetts, New

Jersey, and Pennsylvania. Although considered a primary predator of *A. tsugae* in Japan, it was also collected in a marsh far from hemlock (Sasaji and McClure 1997).

Preliminary laboratory tests showed that *P. tsugae* fed on *A. cooleyi* and *P. strobi* (Cheah and McClure 1996). The host range of *S. ningshanensis* has not been reported, but it has been collected only from hemlock and reared on reproductive *A. tsugae* (Wang et al. 2000). *Scymnus ningshanensis* has been shown in the laboratory to respond numerically to increasing densities of *A. tsugae*, and in caged field studies shown to reduce hemlock woolly adelgid populations (Butin et al. 2003). *Harmonia axyridis* has been shown to be a well-established, generalist predator (Hagen et al. 1999). Here we test the suitability of several adelgid species considered pests in New England and of woolly alder aphid as prey for *P. tsugae*, *S. ningshanensis*, and *H. axyridis* adults.

## Materials and Methods

### Source of Insects

*Pseudoscymnus tsugae* adults were obtained from the Phillip Alampi Beneficial Insect Laboratory, Trenton, New Jersey. *Scymnus ningshanensis* adults were obtained from the USDA Forest Service Insect Rearing Facility, Hamden, Connecticut. *Scymnus ningshanensis* used in the no-choice test experiment were laboratory-reared adults that eclosed in January 1999. *Scymnus ningshanensis* and *P. tsugae* adults used in choice tests were laboratory-reared adults that eclosed in April 2001. We collected *H. axyridis* as pupae, and the beetles eclosed one week before the experiment. Adult beetles were confined in petri dishes for 24 h, in an environmental chamber at 18 °C and 16:8 (L:D) h without food and only water prior to tests.

### No-choice preference tests of *Scymnus ningshanensis*

In 1999 and 2000 we used no-choice tests to determine the feeding preference of *S. ningshanensis* for different taxa of Aphididae as well as *A. tsugae*. We chose aphid species that were found on trees, that were approximately the same size as hemlock woolly adelgid, and that we could identify to species. We compared *S. ningshanensis* consumption of: 1) woolly alder aphid, *Prociphilus tesselatus* Fitch; 2) alder leaf aphid, *Myzocallis alnifoliae* Fitch; and 3) hemlock woolly adelgid using no-choice tests in 1999. In 2000, we used no-choice tests to compare the beetles' consumption of 1) basswood aphid, *Eucalipterus tiliae* (L.); 2) green peach aphid, *Myzus persicae* (Sulzer); and 3) hemlock woolly adelgid.

We placed a 2 cm piece of foliage infested with 10 nymphs of the prey item and one adult coccinellid, starved for 24 h, in a 9 cm petri dish. Adult beetles were allowed to feed for 24 h in a chamber at 18 °C and 16:8 (L:D). After 24 h, we removed the beetles and counted the numbers of each prey species remaining. We replicated each trial at least 16 times for each of the three beetle species tested, all beetle species for each trial were tested the same day, new beetles were used for each trial, and all trials were conducted within the one week. We ran 20 replicates for each prey item tested.

### Choice Tests for Three Coccinellid Species

In 2001, we used choice tests to study the feeding preference of these beetles between prey within the family Adelgidae, and to test the preference between woolly alder aphid and hemlock woolly adelgid. The arenas we used were 9 cm petri dishes with



moistened filter paper on the bottom. A 2 cm piece of eastern hemlock infested with *A. tsugae* and a 2 cm piece of the host plant with the alternate prey item were placed on opposite sides of the dish. We tried to choose pieces of hemlock and alternate host plants that were similar in structure as well as size, so that the amount of foliage on each plant did not influence the beetles' preferences.

In trials comparing hemlock woolly adelgid and an alternate adelgid species, one adult of both species on its host plant were presented in each petri dish. We tested all adelgid species that were available. The trials with non-target adelgids examined coccinellid preferences between hemlock woolly adelgid on its host *T. Canadensis* and either 1) pine bark adelgid (*Pineus strobi* Hartig) on white pine (*Pinus strobus* L.); 2) larch adelgid (*Adelges laricis* Vallot) on larch (*Larix deciduas* Mill); and 3) blue spruce gall adelgid (*Adelges cooleyi*) on Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco).

Another experiment compared coccinellid preferences between hemlock woolly adelgid and woolly alder aphid. On smooth alder, *Alnus serrulata* (Ait.) Willd, foliage, only first instar nymphs of the latter were tested so that the offered alternate prey would be of comparable size. Ten nymphs of each prey species on its host plant were presented in each petri dish.

In each trial, after being starved for 24 h, one adult beetle of a given species was placed in a petri dish. We observed a set of four petri dishes for 15 minutes and recorded each beetle's behavior. We recorded the proportion time spent on the dish, on the hemlock, or on the alternate prey's host plant. Following these observations, we allowed each beetle to feed for 24 h in a chamber at 18 °C and 16:8 (L:D) h. After 24 h, we

recorded the location of each beetle before removing it from the petri dish. At the end of 24 h, the numbers of adelgid adults were counted in the adelgid trials and the numbers of nymphs remaining were counted in the woolly alder aphid trial.

### **Data Analysis**

A one-way analysis of variance was used to analyze differences in consumption of prey species in no-choice tests and the behavioral data from choice tests. Differences among treatment means were tested using Tukey's Pairwise Comparison at  $P = 0.05$ . Chi-square goodness of fit tests were used to analyze feeding and location after 24 h data from choice tests. The statistical analyses of the data were performed using MINITAB<sup>TM</sup> software (Minitab Inc. 2000).

### **Results**

#### **No-choice preference tests of *Scymnus ningshanensis***

The no-choice tests (Table 2.1) showed that *S. ningshanensis* consumed significantly more *A. tsugae* nymphs than all alternate prey items ( $P < 0.05$ ). In addition, *S. ningshanensis* consumed more prey in 1999 than in 2000 ( $P < 0.05$ ).

#### **Choice Tests for Three Coccinellid Species**

Feeding by *P. tsugae* was low in the *A. laricis* and *A. cooleyi* trials (Table 2.2); 0 % and 29 % of the beetles fed on either host when the non-target host was *A. laricis* and *A. cooleyi*, respectively. In the *A. laricis* trial *P. tsugae* adults did not consume adults of either prey species. In the *A. cooleyi* trial, although only 7 beetles fed, and this was only on *A. tsugae* ( $\chi^2 = 32.2$ ,  $df = 1$ ,  $P < 0.05$ ). When the pine bark adelgid was the alternative,

significantly more adults consumed *A. tsugae* ( $\chi^2 = 15.8$ ,  $df = 3$ ,  $P < 0.05$ ). There were significantly more woolly alder aphids (Figure 2.1) than hemlock woolly adelgid remaining ( $F = 8.83$ ,  $df = 45$ ,  $P < 0.05$ ). *Pseudoscymnus tsugae* typically bit one or two woolly alder aphid nymphs, and did not consume whole nymphs. In all trials, *P. tsugae* consumed significantly fewer prey items, including *A. tsugae*, than the other two lady beetle species ( $F = 6.47$ ,  $df = 1$ ,  $P < 0.05$ ).

The behavioral data ( $F = 11.80$ ,  $df = 29$ ,  $P < 0.05$ ) for dishes containing *P. tsugae* (Table 2.3 and Figure 2.2) showed that beetles spent significantly more time on the dish than on any plant species ( $P > 0.05$ ). In all trials there was no significant difference ( $P > 0.05$ ) between the amounts of time beetles spent on each plant species in all trials (Table 2.3 and Figure 2.2).

After 24 h, *P. tsugae* were found significantly more often on hemlock than other locations in the *Adelges cooleyi* (Table 2.4) and woolly alder aphid (Figure 2.3) trials ( $\chi^2 > 5.991$ ,  $df = 2$ ,  $P < 0.05$ ). In the pine bark adelgid trial (Table 2.4) the numbers of beetles found in each location were nearly uniform ( $\chi^2 < 5.991$ ,  $df = 2$ ,  $P > 0.05$ ).

In the *A. laricis* ( $\chi^2 = 33.7$ ,  $df = 3$ ,  $P < 0.05$ ) and *A. cooleyi* ( $\chi^2 = 21.6$ ,  $df = 3$ ,  $P < 0.05$ ) trials (Table 2.2), a significant number of *S. ningshanensis* did not feed on adults of either prey species, but in the *A. cooleyi* trial, beetles that did feed showed no host preference ( $\chi^2 = 5.1$ ,  $df = 2$ ,  $P > 0.05$ ). Beetles equally consumed adults of both adelgid species ( $\chi^2 = 2.36$ ,  $df = 3$ ,  $P > 0.05$ ) in the pine bark adelgid choice test (Table 2.2).

*Scymnus ningshanensis* consumed significantly fewer woolly alder aphid (Figure 2.1) than hemlock woolly adelgid ( $P < 0.05$ ). As in petri dishes containing *P. tsugae*, we noticed *S. ningshanensis* biting woolly alder aphids without consuming whole nymphs.

*Scymnus ningshanensis* spent significantly more time on the dish than on either host plant in all trials ( $F = 17.49$ ,  $df = 29$ ,  $P < 0.05$ ). However, there was no significant difference between the amounts of time spent on hemlock or the alternate host plant (Table 2.3 and Figure 2.2) in any trial ( $P > 0.05$ ).

After 24 h, there was no significant difference in the numbers of *S. ningshanensis* (Table 2.4) found on hemlock or the alternate host plant species in the *A. cooleyi* and pine bark adelgid trials ( $\chi^2 < 5.991$ ,  $df = 2$ ,  $P > 0.05$ ). In woolly alder aphid (Figure 2.2) trial, more *S. ningshanensis* were found on hemlock ( $\chi^2 > 5.991$ ,  $df = 2$ ,  $P < 0.05$ ).

In petri dishes containing *H. axyridis* (Figure 2.1) the ratio for woolly alder aphid remaining was not significantly different than that for hemlock woolly adelgid ( $F = 2.35$ ,  $df = 31$ ,  $P > 0.05$ ). *Harmonia axyridis* consumed nearly 80 % of the woolly alder aphid nymphs and approximately half of the hemlock woolly adelgid nymphs. *Harmonia axyridis* adults spent significantly more time on the dish than on the foliage (Figure 2.2) ( $F = 10.98$ ,  $df = 47$ ,  $P < 0.05$ ), and there was no significant difference between the time spent on hemlock or alder ( $P > 0.05$ ). *Harmonia axyridis* (Figure 2.3) was found principally on the dish in the woolly alder aphid trial ( $\chi^2 > 5.991$ ,  $df = 2$ ,  $P < 0.05$ ).

### Discussion

These preference tests suggest that *P. tsugae* preferred hemlock woolly adelgid to all prey items, including woolly alder aphid. However, it was difficult to draw conclusions about the host range of *P. tsugae* because the beetles fed very little on any

prey species offered. During the behavioral observations, *P. tsugae* adults spent most of the time on hemlock in all but the pine bark adelgid trial. In that trial, equal numbers of adults were

found on white pine and hemlock, which may be because the white pine pieces had slightly denser foliage than other alternate plant items. In these tests the beetles seem to have preferred plants that had foliage under which they could take cover.

In our no-choice tests, *S. ningshanensis* consumed more *A. tsugae* than alternate prey items, suggesting the beetles may prefer adelgids to aphids. In our choice tests, *S. ningshanensis* consumed the adult adelgids, *A. tsugae*, *A. cooleyi*, and *P. strobi* equally, but preferred *A. tsugae* to the aphid, *P. tessellatus* and the adelgid, *A. laricis*. In addition, *Scymnus ningshanensis* explored the environment more than *P. tsugae* and were found equally on most alternate host plants and hemlock. In the woolly alder aphid trial, *S. ningshanensis* explored the alder, but killed very few aphid nymphs.

*Harmonia axyridis* preferred woolly alder aphid to hemlock woolly adelgid, and the beetle spent more time on alder than hemlock. We noticed that *H. axyridis* had difficulty maneuvering through the hemlock needles. Although *H. axyridis* did not prefer *A. tsugae* or hemlock foliage, it was voracious and consumed ca. half of all *A. tsugae* eggs offered.

In all of our trials it was difficult to draw conclusions about the lady beetles' host preferences because all adelgid species continued to lay eggs during the 24 h feeding period. Further host preference studies would be extremely beneficial. If the consumption of eggs is being compared, the adults of all prey species should be removed

prior to the initial egg count. In addition, arenas containing prey species being tested should be set up without the presence of a predator, to serve as a control for each trial.

Well designed host range tests in the laboratory can estimate an insect's feeding preferences, but biological control agents may exploit fewer host species in the field than in the laboratory (Gaugler et al 1997, Sands 1997). All of the adelgids tested in this experiment are exotic and considered pests, and our tests suggest that *S. ningshanensis* or *P. tsugae* seem to prefer hemlock woolly adelgids. However, additional experiments comparing feeding preferences of adults and larvae of each beetle species on alternate prey eggs are necessary before drawing conclusions about non-target impacts of these lady beetles. *Harmonia axyridis* has been shown to feed on several different orders of insects, and this agent might have some ecological consequences to non-target Homopterans such as woolly alder aphid (Ferran et al. 1997, Cottrell and Yeargan 1998, Phoofolo and Obrycki 1998, Abdel-Salam and Abdel-Baky 2001, Yasuda et al. 2001).

## Tables and Figures

Table 2.1: Mean number prey consumed ( $\pm$  SE) in no-choice tests of *S. ningshanensis* adults feeding preference (n = 20 replicates, each with 10 prey).

Prey	Number Prey Consumed	
	1999	2000
Hemlock woolly adelgid	8.6 $\pm$ 0.48	2.5 $\pm$ 0.62
Woolly alder aphid	2.1 $\pm$ 0.55	-
Alder leaf aphid	2.6 $\pm$ 0.83	-
Basswood aphid	-	1.0 $\pm$ 0.51
Greenhouse aphid	-	1.1 $\pm$ 0.45

Table 2.2: Numbers of adult adelgids consumed by lady beetles during 24 given a choice between a non-target adelgid species and *A. tsugae*.

<i>Pseudoscymnus tsugae</i>		
Non-target Species	<i>A. tsugae</i>	n
8 ( <i>P. strobi</i> )	21	27
0 ( <i>A. laricis</i> )	0	26
0 ( <i>A. cooleyi</i> )	7	24
<i>Scymnus ningshanensis</i>		
Non-target Species	<i>A. tsugae</i>	n
14 ( <i>P. strobi</i> )	12	27
0 ( <i>A. laricis</i> )	8	26
4 ( <i>A. cooleyi</i> )	8	26

Table 2.3: Mean time spent (minutes) in each location within the test arena during first 15 min (900 s) of access to choices.

<i>Pseudoscymnus tsugae</i>			
Non-Target Host	On Dish	Hemlock	Non-target
White pine	11.1	2.4	1.5
Larch	7.8	5.3	2.0
Douglas fir	9.5	4.5	1.1

<i>Scymnus ningshanensis</i>			
Non-Target Host	On Dish	Hemlock	Non-target
White pine	12.6	1.4	1.1
Larch	9.8	4.2	1.1
Douglas fir	8.3	3	3.4

Table 2.4: Number of beetles found in each location after feeding for 24 h.

<i>Pseudoscymnus tsugae</i>			
Non-Target Host	On Dish	Hemlock	Non-target
White pine	5	11	9
Larch	0	20	6
Douglas fir	1	17	6

<i>Scymnus ningshanensis</i>			
Non-Target Host	On Dish	Hemlock	Non-target
White pine	8	10	7
Larch	1	22	3
Douglas fir	2	11	13



Figure 2.1: Number prey consumed (mean  $\pm$  SE) after 24 h in the choice test between hemlock woolly adelgid nymphs and woolly alder aphid nymphs.

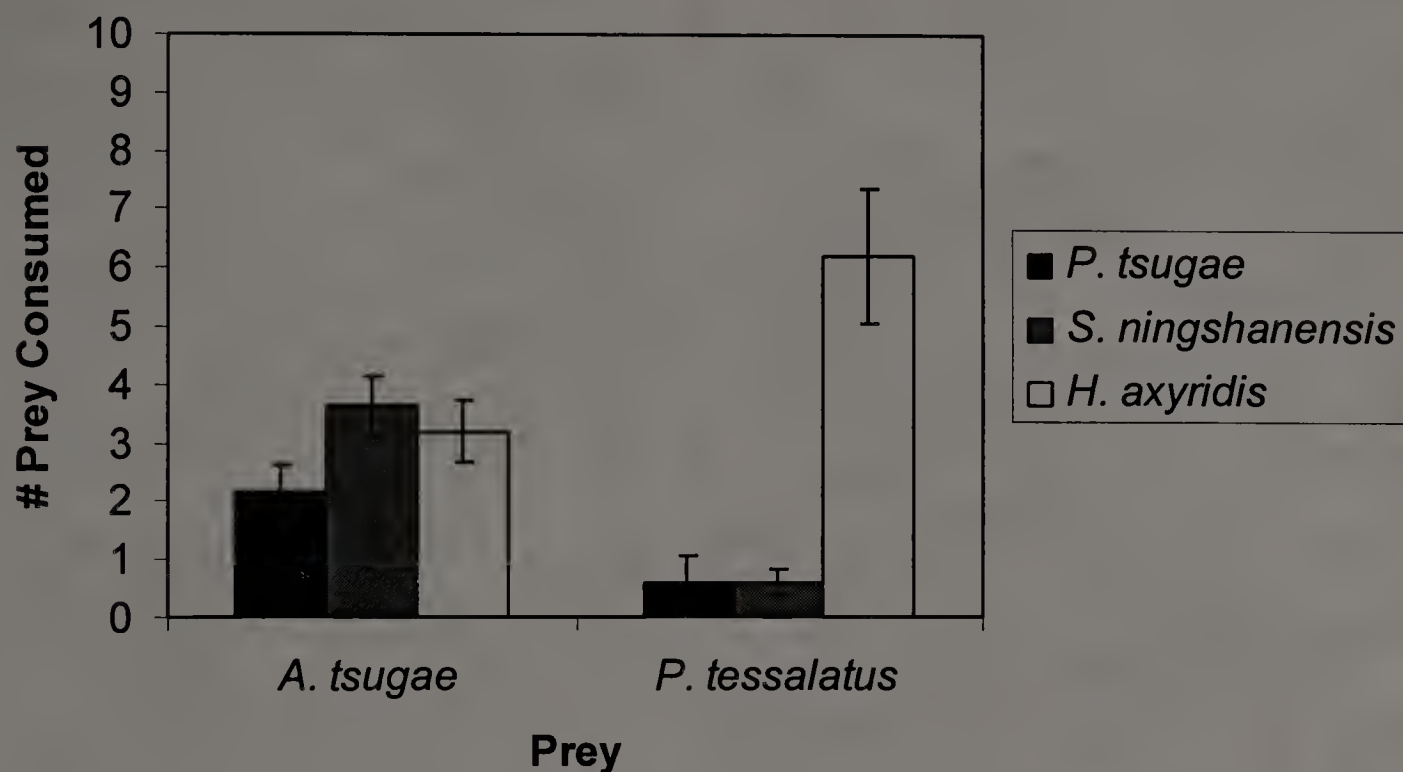


Figure 2.2: Mean time spent (mean  $\pm$  SE) on foliage with hemlock woolly adelgid or woolly alder aphid during the first 15 min (900 s) of access to the choices.

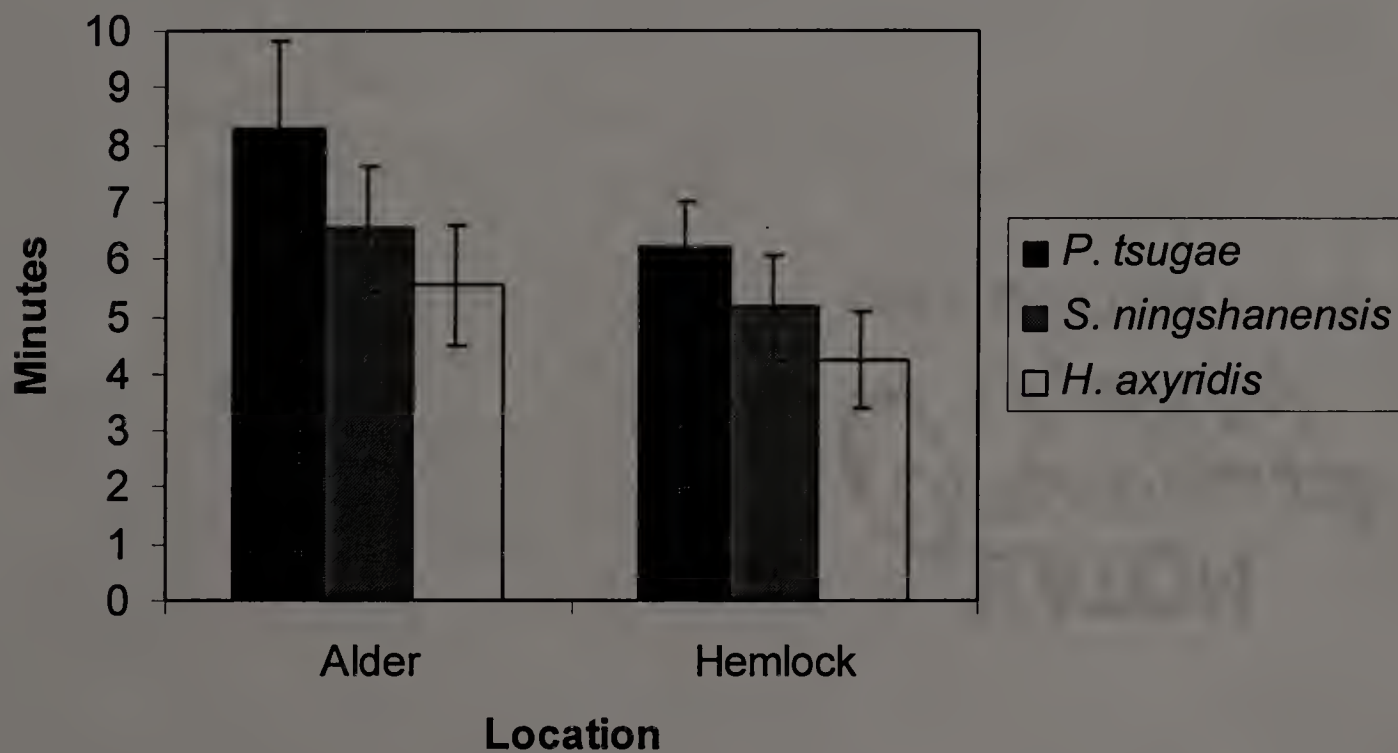
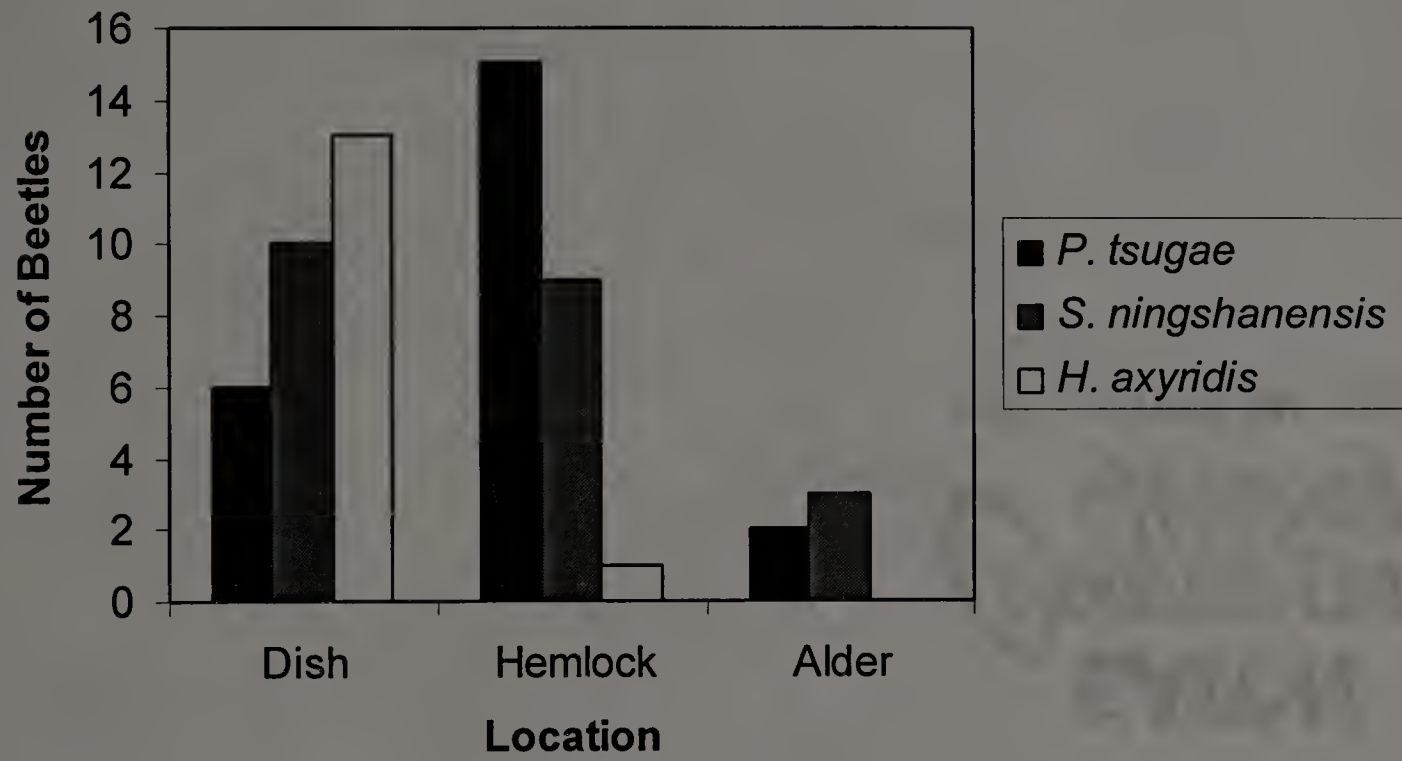


Figure 2.3: Numbers of beetles found in each location after feeding for 24 h.



## CHAPTER 3

### EVOLUTION OF COLD-HARDINESS IN THE HEMLOCK WOOLLY

#### ADELGID (HOMOPTERA: ADELGIDAE)

##### Introduction

Invasive species can cause severe ecological and economic impacts (Simberloff 1996). Population biology studies such as phylogeographic structure, ecology, life-history, and evolution of a successful invasive species can provide insight into the understanding and management of detrimental invasive species (Sakai et al. 2001).

Some exotic species are more successful invaders than others (Bazzaz 1986). One reason may be that the new environment is more favorable than native habitats because of escape from natural enemies and from competitors (Kaufman and Smouse 2001). Successful invaders may also be more ecologically dominant if they are parthenogenic. Since reproductive success does not depend upon the location of mates, reproduction can occur during harsh circumstances (Novak et al. 1991). Finally, successful invasive species are believed to have life-history traits that predispose them to be effective invaders. These traits can include small body size, high fecundity, short maturation time, and phenotypic plasticity (Kolar and Lodge 2001).

Here we use the hemlock woolly adelgid as an example of a successful invasive species. Hemlock woolly adelgid was introduced in the 1950s and has been expanding its range northward, to the detriment of hemlock trees in the eastern United States.

The hemlock woolly adelgid, *Adelges tsugae* Annand, is an exotic pest from Asia that has extensively damaged hemlocks in the eastern United States (Orwig and Foster

1998). The first reported confirmation of *A. tsugae* in North America was on *Tsuga heterophylla* Sargent in California, Oregon, and British Columbia in the 1920s (Annand 1928). The hemlock woolly adelgid was later recorded on eastern hemlock (*Tsuga canadensis* Carriere) and Carolina hemlock (*Tsuga caroliniana* Englemann) in Virginia in 1951, and was discovered in Connecticut in 1985 (Anonymous 1968, McClure 1997). Hemlocks have an important role in watershed ecosystems because they have strong effects on microclimate and soil conditions, and they provide a distinctive habitat for certain wildlife species (Orwig and Foster 1998). Since *A. tsugae* is prolific and native natural enemies have not yet adequately controlled *A. tsugae*, adelgid populations can increase rapidly and cause severe dieback to eastern and Carolina hemlocks in the United States (Montgomery and Lyon 1996).

In the United States, *Adelges tsugae* is parthenogenic with an elaborate life cycle. In New England the sistens (winter generation) begin oviposition in March. These eggs hatch into progrediens (summer generation) that develop into one of two morphs. The winged progrediens in Asia fly to spruce (*Picea* spp.) and reproduce sexually, but in North America there are no spruce species suitable for the hemlock woolly adelgid. The wingless progrediens remain on hemlock and begin oviposition in May. These eggs hatch into sistens that enter aestivation in August and resume development in October (McClure 1989).

*Adelges tsugae* sistens adults are approximately 1.4 mm long and 1.1 mm wide, while the progrediens adults are 0.9 mm long and 0.6 mm wide. Each female sistens lays

a mean of 50 eggs, but some females can lay up to 300 eggs in a single season.

*Progrediens* lay a mean of 22 eggs, and some can lay up to 250 eggs in one season. There are two generations per year (McClure 1989).

The current range of the hemlock woolly adelgid (Figure 3.1) is from North Carolina to coastal New Hampshire in the eastern United States, while the native range of hemlocks (Figure 3.1) in this area is from Georgia to southern Canada (Godman and Lancaster 1990). McClure (1990) found that hemlock woolly adelgids can be spread in the egg or crawler stage by both wind and birds, and adelgids were found as far as 600 m from the infested site. The spread of *A. tsugae* northward from its current distribution may be limited by its ability to survive winter temperatures typical of plant cold-hardiness zones north of USDA Zone 5, where average annual minimum temperatures range from  $-20^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  (Parker et al. 1998).

Past experiments conducted on *A. tsugae* cold-hardiness have shown that mortality increases as temperature of the cold treatment decreases. Also, adelgid cold-hardiness decreases as the adelgids mature. *Adelges tsugae* collected in January and February survived sub-zero temperatures better than adelgids collected in March (Parker et al. 1998, 1999). Exposure to sub-zero temperatures causes mortality by destroying cells found in adelgid hemolymph (Parker et al. 2000). However, these authors noted that in all cold treatments there was always at least ten percent adelgid survival (Parker et al. 1998). This suggests that sub-zero temperatures may select for cold tolerance in *A. tsugae*.

Here we explore the possibility that *A. tsugae* has evolved the ability to survive colder temperatures as it gradually spread northward from Virginia. We compare the

cold-hardiness of *A. tsugae* from the southern and northern edges of its distribution in 2001 and 2002, and determine if differences are due to maternal effects, genetic variation, or phenotypic plasticity.

## Materials and Methods

### **Comparing Two Populations of *A. tsugae***

Adelgid-infested twigs were collected from three sites in Maryland (Annapolis, Gaithersburg, and Clarksburg) and three sites in Massachusetts (Springfield, Holyoke, and Amherst). In mid-January 2000 we collected 15 infested twigs (15 cm in length) from each location. We counted the numbers of live sistens nymphs on each twig, and the twigs were held in florist foam in a cooler at 2°C for one month. We randomly selected 10 infested twigs from each site, exposed them to a cold snap for 36 hours in a freezer at -15°C. We chose this temperature regime because a previous attempt to cold-shock the adelgids at -15°C for 72 hours resulted in 100 percent mortality of adelgids from both states. By cutting the exposure time in half we hoped to reduce the mortality by approximately 50 percent. After the twigs were cold-shocked, we moved them back to the cooler at 2°C. The five remaining twigs stayed in the cooler at 2°C for the duration of the experiment. After one week we counted the numbers of living sistens on all twigs. Each twig represented one replicate.

## Comparing offspring of *A. tsugae*

We controlled for maternal effects on hemlock woolly adelgid cold-hardiness by rearing offspring from Massachusetts and Maryland adelgids in the same environment for a generation. We used the infested twigs collected in January 2001 (ones that were not subsequently exposed to a cold snap in 2001) to rear *A. tsugae* offspring. Beginning in March 2001, these twigs were exposed to a thermal regime consisting of temperature increases at increments of 5°C every two weeks until the temperature reached 15°C. The sistens on the twigs from Gaithersburg, MD, Clarksburg, MD, Springfield, MA, and Amherst, MA successfully oviposited. In May 2001, each twig was used to infest a healthy, uninfested hemlock branch on trees in Massachusetts. We enclosed each branch in a 30 cm<sup>2</sup> nylon mesh bag with a weave tight enough to trap the adelgids, but still allow for ventilation.

Branches were collected the following year (January 2002). We cut each branch into 20, 15 cm twigs and counted the numbers of live sistens on each twig. We randomly selected 15 twigs from each branch, put them in a freezer at -15 °C, and after 36 h moved them back to the cooler at 2° C. The five remaining twigs stayed in the cooler at 2 °C. After one week we counted the numbers of living sistens on all twigs.

## Data Analysis

Logistic regression was used to analyze treatments within and between Massachusetts and Maryland. The equation for the regression model for comparing treatments within a state was  $\ln(P/1-P) = \beta_1(\text{treatment}) + \beta_0$ , where  $P$  is the percent

mortality. The equation for the regression model when we compared states for one treatment was  $\ln(P/1-P) = \beta_1(state) + \beta_0$ . The statistical analyses were performed using JMP software (SAS Institute 1995).

## Results

### **Source Populations**

There was significantly higher mortality (Figure 3.2) of the adelgids from Maryland that were cold-shocked compared to cold-shocked adelgids from Massachusetts ( $\chi^2 = 93$ ,  $df = 1$ ,  $P < 0.05$ ). The mean percent mortality of adelgids collected in Maryland was 68 percent ( $\pm 2.4$  SE) versus 58 ( $\pm 2.5$  SE) percent in Massachusetts. The mean percent mortality of adelgids that were not cold-shocked (Figure 3.2) was also significantly different ( $\chi^2 = 57.3$ ,  $df = 1$ ,  $P < 0.05$ ), with adelgid mortality from Maryland being lower than Massachusetts.

### **Offspring of Source Populations**

The mean percent mortality of cold-shocked offspring (Figure 3.3) whose mothers were from Maryland was 82 ( $\pm 2.6$  SE) percent. This mortality was significantly higher than the cold-shocked offspring from Massachusetts 61 percent ( $\pm 5.0$  SE) ( $\chi^2 = 34$ ,  $df = 1$ ,  $P < 0.05$ ). At the control temperature (2 °C), adelgids whose mothers were from Massachusetts (Figure 3.3) suffered 17 ( $\pm 5.7$  SE) percent mortality, whereas those from Maryland suffered 7.5 ( $\pm 5.6$  SE) percent mortality. These mortalities were not significantly different.



When comparing sites within each state, the mean percent mortality of cold-shocked offspring from Clarksburg, MD (Figure 3.4) was significantly lower than cold-shocked offspring from Gaithersburg, MD ( $\chi^2 = 57, df = 7, P < 0.05$ ). Within Massachusetts (Figure 3.5) the mean percent mortality of cold-shocked offspring from Amherst was significantly higher than from Springfield ( $\chi^2 = 7, df = 7, P < 0.05$ ). The percent mortalities at the control temperature were not significantly different between either site in Maryland or Massachusetts.

Adelgids from both states survived better when they were not cold-shocked Table 1). There was a significant state effect (Table 1); the total average mortality was higher among adelgids collected in Maryland than in Massachusetts. Given the common rearing environments, this suggests that geographic variation of the adelgid has evolved between the two states. There was also significant within-state variation (Table 1). The total average mortality was higher among adelgids collected in Gaithersburg, MD than in Clarksburg, MD, and the total average mortality was higher among adelgids collected in Amherst, MA than in Springfield, MA. This effect suggests that geographic variation of the adelgid has evolved within each state as well. Finally, there were significant treatment by state and treatment by location (within state) effects (Table 1). These effects suggest that the adelgids from each state (Figure 3.3) and from each location (Figures 3.4 and 3.5) are responding differently to the treatments due to gene by environment interaction and genetic differences.

## Discussion

Our data suggest that *A.s tsugae* evolved cold-hardiness as it expanded its range from Virginia to Massachusetts in a little more than 100 adelgid generations. We wonder how hemlock woolly adelgid evolved so quickly given that the insects are clonal.

There are several ecological characteristics that may have influenced the success *A. tsugae*. *Adelges tsugae* may be successful because its life-history traits match those that Kolar and Lodge (2001) suggest make an invader successful. Hemlock woolly adelgid is an “r-selected” species; it is small in size; it has a high fecundity; and it is multivoltine (McClure 1989). Perhaps more importantly, hemlock woolly adelgid is strictly parthenogenic in North America (McClure 1989). Hemlock woolly adelgid escapes the allee effect (Allee 1931) because population growth in small populations is not positively density dependent on population size. Keitt et al. (2001) showed that Allee effects can influence the rate of range expansion. Populations near the edge of invasions are often low, so the proximity of mates influences the rate of spread (Keitt et al. 2001). However, species like *A. tsugae* that are parthenogenic, and therefore do not rely on the proximity of mates, are often good colonists (Novak et al. 1991).

The genetic variation of cold-hardiness between and within Maryland and Massachusetts may also be the result of genetic and evolutionary processes. Evolution of cold-hardiness may have occurred during initial colonization or during range expansion when individuals encountered new selection pressures (Travis and Dytham 2002). The genetic variation could be due to mutation, considering the large population sizes hemlock woolly adelgid can achieve. A more likely source of genetic variation was

variation that existed in the founder population. A large initial population of adelgids may not have been required to represent the genetic variability that existed in a larger source population in Asia.

Lag times are often a trait of invasions and can occur between an invader's initial colonization and the beginning of population growth and range expansion. Lag times are expected if evolutionary processes are necessary for successful colonization (Ellstrand and Schierenbeck 2000). The probability of an invader being successful seems to depend upon genetic constraints. Lag times could be due to the time required to overcome genetic constraints such as, evolution of characteristics for adaptation in a novel environment or avoidance of inbreeding depression (Mack et al. 2000).

There was a significant lag time from when the initial colonies of *A. tsugae* were found in Virginia and when they were reported in New York and Connecticut. Hemlock woolly adelgid was introduced in Virginia in 1951, and over the following 30 years *A. tsugae* spread through the mid-Atlantic states. Hemlock woolly adelgid was reported in Pennsylvania in 1960 in New York in 1980, and in Connecticut in 1986 (Souto et al. 1996). Before the 1960s *A. tsugae* was considered a nuisance pest on ornamentals, but when the insects infested native hemlocks, the rate of spread and impact of the hemlock woolly adelgid changed. Then in 1985, Virginia experienced a severe cold snap. Hemlock woolly adelgid populations were reduced, but not completely wiped out. *Adelges tsugae* populations from Virginia to Connecticut were again reduced during a cold snap in 1993, but populations steadily recovered (Souto et al. 1996).

*Adelges tsugae* is an excellent example of an invasive insect species that has the appropriate life-history traits to evolve and continue to invade novel environments. We

found that adelgids from Massachusetts are genetically different than those from Maryland. We also found that there is genetic variation of cold-hardiness of hemlock woolly adelgids within each state. The genetic variation within Massachusetts and Maryland indicates a propensity for hemlock woolly adelgid to further evolve cold-hardiness and expand its range into northern New England.

## Tables

Table 3.1: Results from the logistic regression of the offspring data. () = nested, \* = interaction

<u>Source</u>	<i>df</i>	$\chi^2$	<b>P</b>
Treatment	1	225	0
State	1	7	0.001
Location(State)	2	64	0
Treatment*State	2	7	0
Treatment*Location(State)	2	34	0.3

## Figures

Figure 3.1: Range expansion of *Adelges tsugae* in the eastern United States (Map courtesy of USDA Forest Service website)

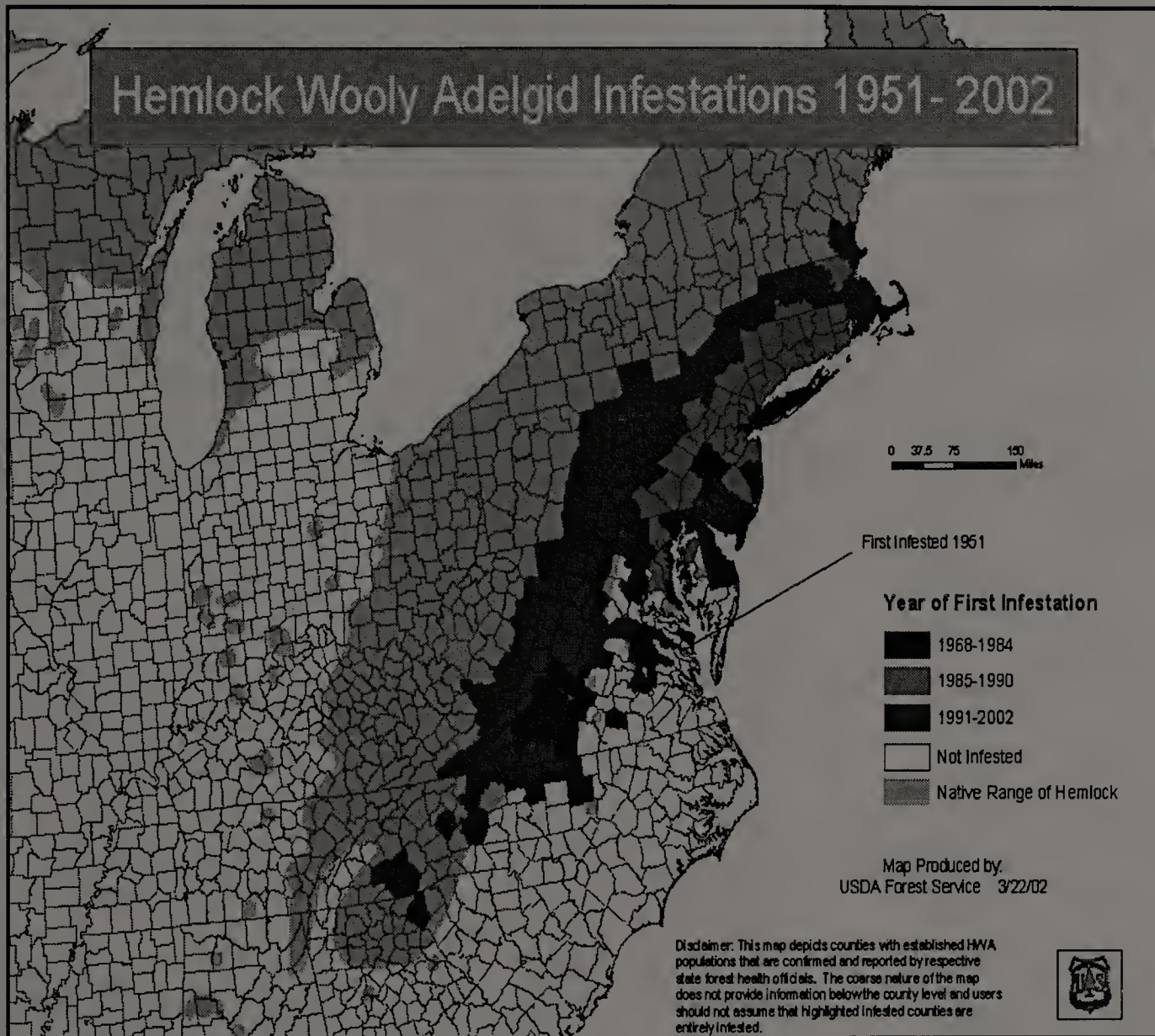


Figure 3.2: Average percent mortality of *Adelges tsugae* collected in January 2001 from Maryland and Massachusetts and exposed to -15 °C (cold-shocked) or 2 °C (control).

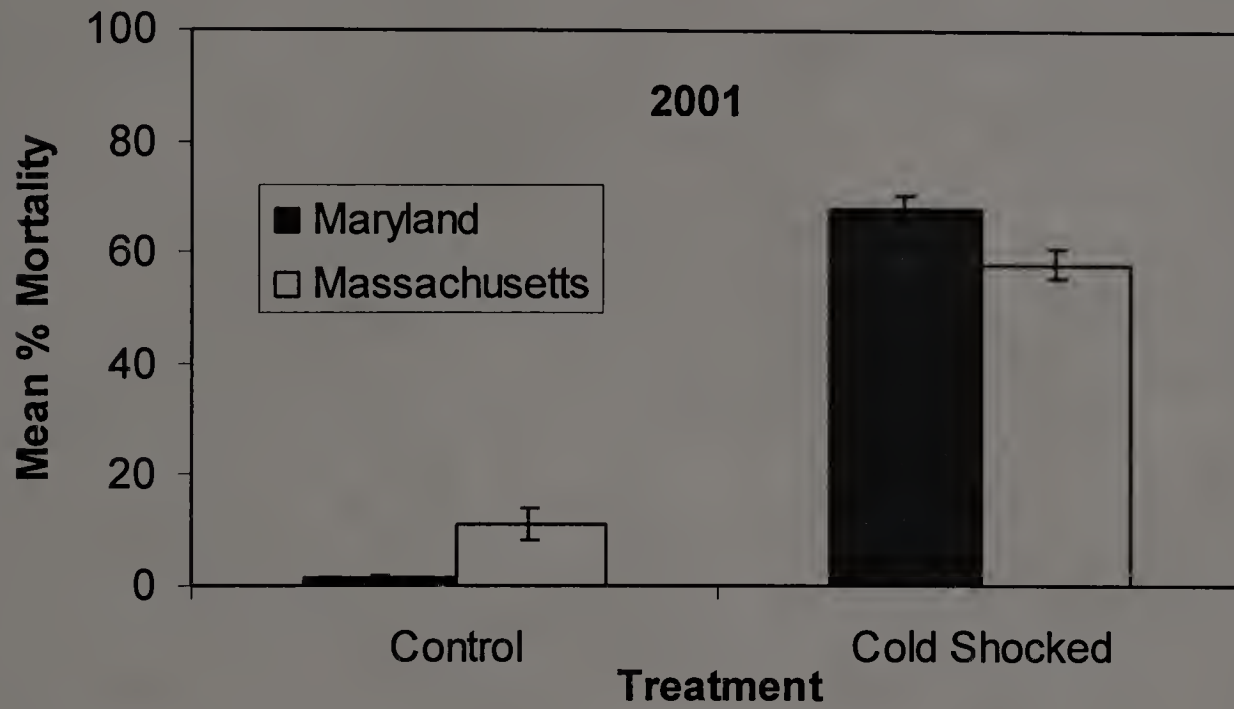


Figure 3.3: Average percent mortality of *Adelges tsugae* offspring reared in the same environment whose mothers were from Maryland or Massachusetts; offspring were exposed to either -15 °C (cold-shocked) or 2 °C (control).

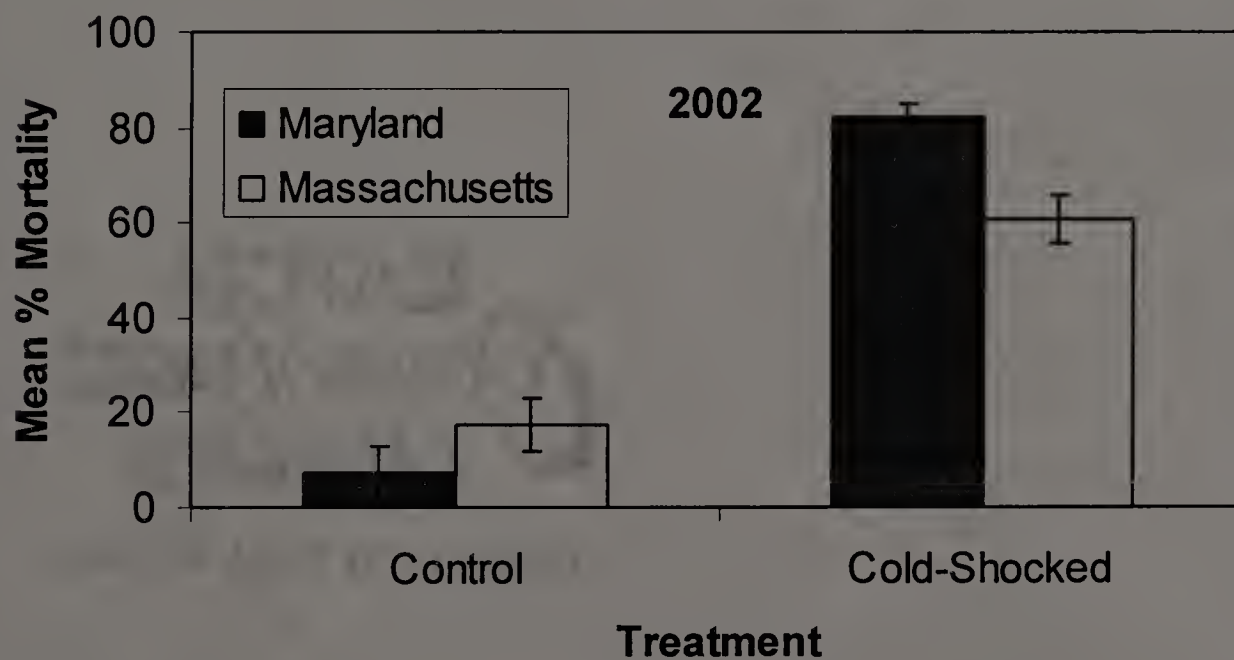


Figure 3.4: Average percent mortality of *Adelges tsugae* offspring reared in the same environment whose mothers were from Clarksburg, Maryland or Gaithersburg, Maryland; offspring and were exposed to either -15 °C (cold-shocked) or 2 °C (control).

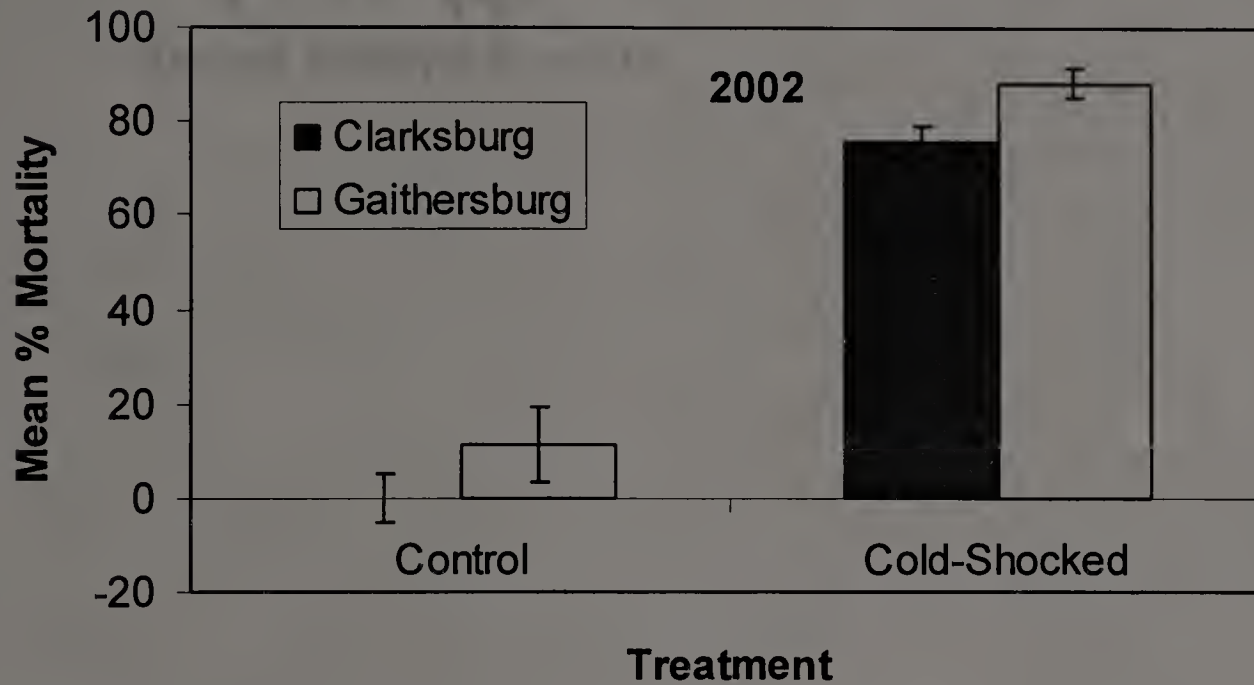
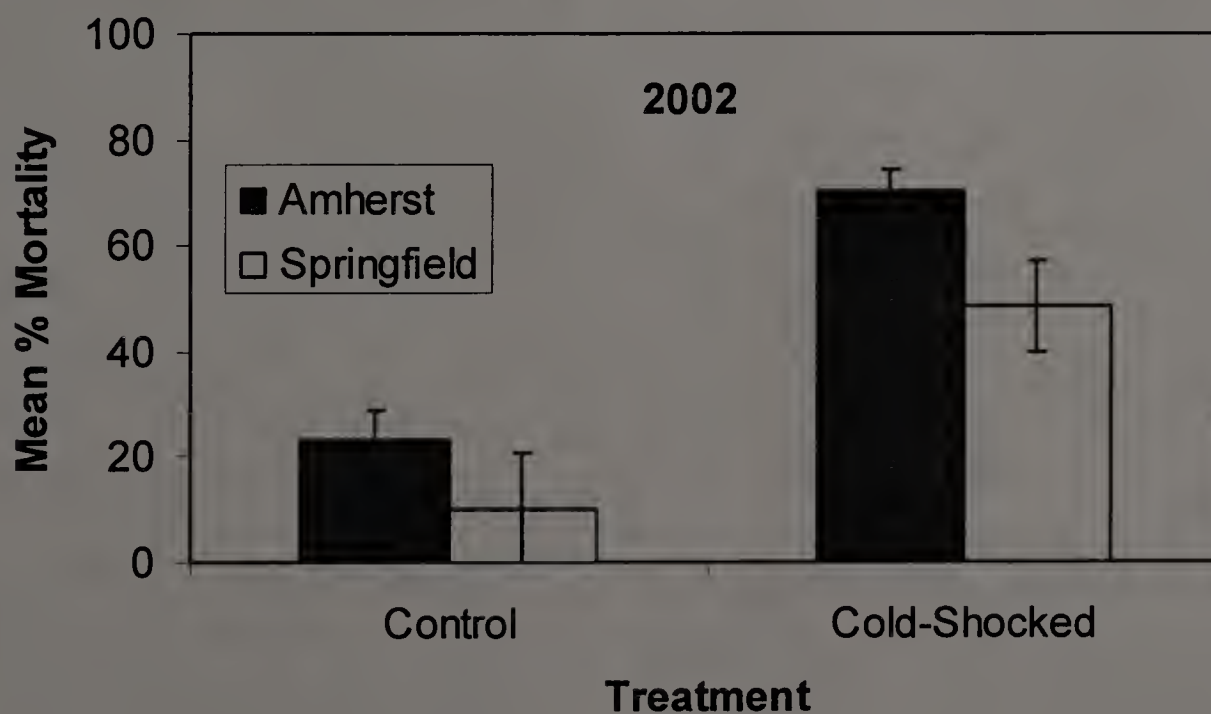


Figure 3.5: Average percent mortality of *Adelges tsugae* offspring reared in the same environment whose mothers were from Springfield, Massachusetts or Amherst, Massachusetts; offspring and were exposed to either -15 °C (cold-shocked) or 2 °C (control).





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