Pre-release Host Range Assessment for Classical Biological Controls: Experience with Predators for the Hemlock Woolly Adelgid

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Abstract

There are few regulations for the release of parasitoids and predators, compared to herbivorous arthropods and pathogens, used as classical biological control agents in the United States The types of tests conducted prior to release of the predator or parasitoid into the environment are often up to the individual wishing to introduce the new agent. Ethical researchers understand the importance of host range testing to examine non-target impacts before releasing exotic arthropods into the environment. We use classical biological control of the hemlock woolly adelgid (HWA), *Adelges tsugae*, and host range testing of natural enemies for control of HWA as an example. The hemlock woolly adelgid is an exotic pest native to Asia that can cause severe damage to hemlocks in the eastern United States Several natural enemies have been imported, and two agents with good potential are the coccinellids, *Pseudoscymnus tsugae* from Japan and *Scymnus ningshanensis* from China. We included *Harmonia axyridis* in the feeding preference trials to show the non-target impacts of a generalist predator. The host range tests for *P. tsugae* were inconclusive because the beetles ate significantly fewer prey items, including HWA, than the other beetle species; but this species seemed to prefer HWA over other adelgids and aphids. *Scymnus ningshanensis* preferred hosts within the family Adelgidae over Aphididae or other alternate prey items ($p < 0.05$). *Harmonia axyridis* was the only species that consumed more woolly alder aphid nymphs than HWA nymphs. *Scymnus ningshanensis* and *P. tsugae* have narrow host ranges in the laboratory and may be more host-limited in the environment, so releases of the beetles may have few ecological consequences. *Harmonia axyridis*, however, is a known generalist and mass releases of the beetles are known to have negative impacts on non-targets.

Keywords:

Adelges tsugae, *Pseudoscymnus tsugae*, *Scymnus ningshanensis*, *Harmonia axyridis*, host range testing.

Introduction

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an exotic pest native to Asia. HWA was first reported on hemlocks in Oregon in the early 1920s (Annand 1924), where hemlock species are resistant to the insect. In 1951 HWA was found on hemlocks in Virginia (Anonymous 1968), and HWA can reach injurious levels on *Tsuga caroliniana* Englemann and *T. canadensis* Carriere in the eastern United States (Orwig and Foster 1998).

Control options of HWA in natural forested environments are limited. Chemical and cultural controls are not feasible in natural stands (Cheah and McClure 1998). Native natural enemies have not held HWA below injurious levels (Montgomery and Lyon 1996). Classical biological control, or the collection, importation, and release of natural enemies into new areas, has the most potential of all control strategies. There are ecological consequences to be concerned about when introducing an exotic natural enemy. The first major consequence is failure of the new biological control agent to establish, which can be the result of many different factors. The second major consequence is the interaction of the new biological control agent with non-targets. These interactions include the new control agent predating upon or competing with non- targets, resulting in extinctions. Host range testing can provide information on the biological control agent's feeding specificity (Van Driesche and Bellows 1998).

Classical Biological Control Regulations in the United States

The regulations for importing natural enemies (predators, parasitoids, herbivores, and pathogens) of plant pests or weeds are similar. A PPQ FORM 526 must be submitted to Animal and Plant Health Inspection Service (APHIS), and upon approval the potential biological control agent must be transported into an approved quarantine facility. While in the quarantine four main questions must be answered. Does the new biological control agent harbor contaminants? Does the new biological control agent feed on plants? What is the biological control agent's biology? Has the biological control agent's identity been confirmed? (APHIS 2002).

Regulations for removal from quarantine, however, are quite different between herbivorous organisms and entomophagous organisms. To remove herbivorous arthropods from quarantine, such as biological controls for weeds, APHIS has created stringent regulations, including guidelines for host range testing. The requirements to remove parasitoids or predators from quarantine are less rigorous. For organisms that do not feed or otherwise cause direct harm to plants, another PPQ FORM 526 is submitted to APHIS with "additional information." This information should include the proposed action, biological control agent information, target pest information, and environmental and economic impacts of the agent. The required host range testing information may be limited to data from literature, museum records, or unpublished data. APHIS's concern about the new natural enemy's impact on non-targets is limited to commercially important species, such as bees that pollinate crop plants. There are no specific guidelines regarding host range testing. Generally, APHIS will grant removal from quarantine and send a letter of "no jurisdiction" if the identity of the predator or parasitoid is known, if it has been shown not to feed on plants, and if it is free of contaminants (APHIS 2002).

Regulations regarding release into the environment also depend on whether the organism feeds or otherwise directly affects plants. Environmental release of herbivorous arthropods or pathogens is regulated by APHIS. Release of pathogens attacking insects also is subject to review by APHIS if the use is limited to less than10 acres (regulation is by the Environmental Protection Agency when applied to more than 10 acres). For pathogens under its jurisdiction and herbivorus arthropods, APHIS will conduct an environmental assessment and prepare documents required under the National Environmental Protection Act of 1969 (NEPA) and the Threatened and Endangered Species Act of 1973. The environmental release of predators or parasitoids of plant pests is not regulated by APHIS; however, there may be other federal restrictions on releasing them into the environment.

If the release involves federal employees or agencies, occurs on federal land, or involves the use of federal funds, the guidelines for environmental assessment (EA) under NEPA should be followed. The EA is written using guidelines the cooperating federal agency (e.g., USDA Forest Service) has created, which are general and have no specific requirements, such as host range testing for examining biological controls. The EA is used to notify the public of the release, and provides an opportunity for the public to review and comment on the proposed action. After the EA is submitted, a director of the cooperating federal agency (may be delegated to a lower level administrator such as a project leader) determines if the release will significantly affect the human environment. If it is determined that the release may have a negative impact, a more detailed and broader environmental impact statement (EIS) is prepared for review by the public and other federal agencies. If a determination is made that the release will not affect the human environment a finding of no significant impact (FONSI) is created.

Even though the release of the organism may not be subject to federal regulation, ethical researchers will obtain an understanding of the ecological consequences of their new biological control agent before releasing it into the environment. The types of tests conducted (including host range testing) on the predator or parasitoid prior to release, and whether the natural enemy should be released, becomes a judgment call. Ideally, the researcher would like to answer in the laboratory questions such as: "Will the new natural enemy adequately control the target pest?" or "What types of non-targets might the natural enemy affect?" The first question can be addressed by conducting numerical and functional response experiments. The second question can be addressed by conducting host range tests.

Classical Biological Control of HWA: A Case Study

Several biological control agents have been imported for control of HWA (Cheah and McClure 1996). The two agents with the most potential are *Pseudoscymnus tsugae* Sasaji and McClure, a lady beetle from Japan already being released in North America (McClure 2000) and *Scymnus ningshanensis* Yu at Yao, a lady beetle from China that is a candidate for release (Montgomery et al. 2000). The host ranges of *P. tsugae* and *S. ningshanensis* have not been documented. Therefore, we conducted host range tests to determine the coccinellids' feeding preferences.
We used no-choice tests to determine the range of different taxa the predators will eat when in a starved condition. In this situation, non-consumption of an offered food is a strong indication that it

will not be consumed in nature; however, one cannot conclude that a food that is consumed would be eaten in nature. Choice-tests were used to provide a better idea of host specificity within closely related taxa and which foods would be preferred in nature.

No-Choice Tests of Non-Target Species. Adults of *S. ningshanensis* were tested in the no-choice trials. We compared the beetles' consumption of the woolly alder aphid, *Prociphilus tesselatus* Fitch; an alder leaf aphid, probably *Myzocallis alnifoliae* Fitch; and HWA using no-choice tests in 1999. In 2000, we used no-choice tests to compare the beetles' consumption of the basswood aphid, *Eucalipterus tiliae* (L.); a greenhouse aphid, probably *Myzus persicae*; a psocid on hemlock; and HWA. All of the species tested, except the psocids, are considered plant pests in the United States. The psocids feed on algae, fungi, pollen, dead insects, etc. and are not harmful to trees. Woolly alder aphid is one of the primary hosts of larvae of the harvester butterfly, *Feniseca tarquinius* (Fabricius), the only carnivorous Lepidoptera species in North America. Environmental NGO (nongovernmental organizations) were concerned about impacts of the harvester butterfly's food source, so we responded by comparing feeding rates of the lady beetles on woolly alder aphid.

Methods. We placed a 2-cm piece of foliage infested with 10 nymphs of the prey item in a 9-mm petri dish. One adult coccinellid starved for 24 hours was placed in the dish. Adult beetles were allowed to feed for 24 hours in the petri dishes, and dishes were kept in a chamber at 18^{o}C and 16:8 (L:D). After 24 hours we removed the beetles and counted the numbers of each prey species remaining. We used 20 replications for each prey item tested.

Results and Discussion. The no-choice tests showed that *S. ningshanensis* preferred HWA to the other prey items (Table 1). *Scymnus ningshanensis* adults consumed significantly more HWA nymphs than all other prey items offered ($P < 0.05$, two sample t-test, Minitab 12.0).

Table 1. Numbers of Prey Consumed by *Scymnus ningshanensis* in No-Choice Feeding Test (24 Hours).

Prey	No. Eaten
Hemlock woolly adelgid	8.6
Woolly alder aphid	2.1
Alder leaf aphid	2.6
Basswood aphid	1.0
Greenhouse aphid	1.1
Hemlock psocid	0

Choice Tests of HWA and Non-Target Species. We conducted choice test trials between species of adelgid to determine the feeding preferences of both lady beetles. Two adelgid species were compared to HWA: larch adelgid, *Adelges laricis* Vallot, on larch, *Larix laricina* (Du Roi) K. Koch; and pine bark adelgid, *Pineus strobi* Hartig, on white pine, *Pinus strobus* L. Both adelgid species tested are exotic and are considered pests. In a third trial we tested the lady beetles' consumption of woolly alder aphid, *P. tesselatus*, vs. HWA.

We also did choice tests with the woolly alder aphid and HWA, which included *Harmonia axyridis* Pallas. This lady beetle species is considered a nuisance because adults aggregate in buildings in the fall to hibernate. Also, this species can feed and develop on many Homoptera, and also has been shown to feed on native coccinellid species (Cottrell and Yeargan 1998; Yasuda et al. 2001), native lacewings (Phoofolo and Obrycki 1998), and eggs of at least two species of Lepidoptera (Abdel-Salam and Abdel-Baky 2001; Ferran et al. 1997). We observed *H. axyridis* adults and larvae feeding on woolly alder aphid in the field and adults feeding on *S. ningshanensis* larvae in our laboratory.

Methods. Numbers of eggs present on a 2 cm piece of foliage were counted in the adelgid trials, and the numbers of nymphs present were counted for woolly alder aphid versus HWA on a 2 cm piece of foliage. No adelgid eggs were present in the latter trial since the aphid gives birth to live young. We also made sure that only the smallest, first instar aphids were used, so that size of prey would not be a factor. The two pieces of foliage were placed on opposite sides of a 9-mm petri dish. We placed one adult coccinellid starved for 24 hours in a dish, observed it for 15 minutes, and recorded the amount of time the beetle spent on the dish, the hemlock, or the alternative prey. Adult beetles were allowed to feed for 24 hours in the petri dishes, and dishes were kept in a chamber at 18^{o}C and 16:8 (L:D). After 24 hours we recorded the location of each beetle (locations were the same as those used in the observations). We removed the beetles and counted the numbers of each prey species remaining. In the larch adelgid trial there were more adelgid eggs after 24 hours, so the proportion of prey remaining was calculated rather than the proportion of prey consumed. We used 20 replications of each prey choice for each beetle species.

Results and Discussion. In the comparisons with larch adelgid (Figure 1), the proportion HWA remaining was significantly lower than the proportion of larch adelgid both in dishes containing *P. tsugae* and in dishes containing *S. ningshanensis* ($P < 0.05$, two sample t-test, Minitab 12.0). *Pseudoscymnus tsugae* adults spent most of the time on the dish ($P < 0.05$, ANOVA, Minitab 12.0) and more time on hemlock than on the alternate host plants in the larch adelgid trial, and after 24 hours significantly more ($P < 0.05$, chi square, Minitab 12.0) beetles were found on hemlock than on larch. *Scymnus ningshanensis* explored the petri dish more than *P. tsugae*. Adults spent equal amounts of time on hemlock and larch adelgid during the first 15 minutes, but after 24 hours more beetles were found on hemlock than on larch ($P < 0.05$, chi square, Minitab 12.0).

In the comparisons with pine bark adelgid (Figure 2), there was no difference between the proportion HWA and PBA eggs remaining in petri dishes containing either *P. tsugae* or *S. ningshanensis* ($P < 0.05$, two sample t-test, Minitab 12.0). The amount of time during the first 15 minutes both beetle species spent on white pine and hemlock were not significantly different, and after 24 hours equal numbers of beetles ($P > 0.05$, chi square, Minitab 12.0) were found on hemlock and white pine.

In choice tests between HWA and the woolly alder aphid (Table 2), *P. tsugae* and *S. ningshanensis*, after 24 hours, consumed significantly fewer woolly alder aphids than HWA ($P < 0.05$, two sample t-test, Minitab 12.0). During the first 15 minutes, we observed that both *P. tsugae* and *S. ningshanensis* nibbled on one or two woolly alder aphid nymphs, yet no nymphs were consumed entirely. *Hsrmonia axyridis* preferred woolly alder aphid to HWA ($P < 0.05$, two

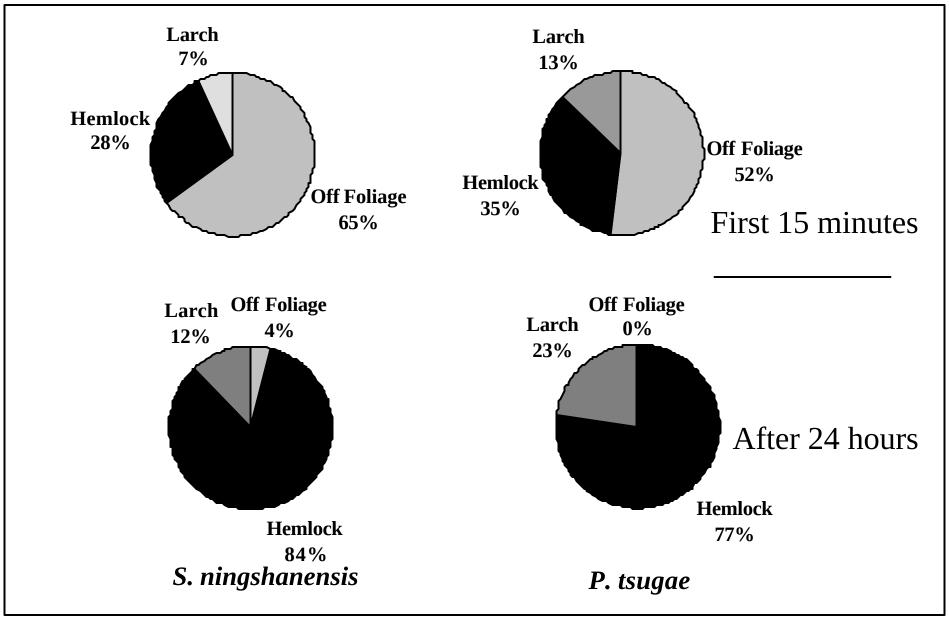

Figure 1. Location of adult lady beetles given a choice of adelgid-infested hemlock or larch foliage in a petri dish arena. Percentages are based on time spent at each location during the first 15 minutes following introduction of a beetle and the number of beetles at each location after 24 hours (N = 25).

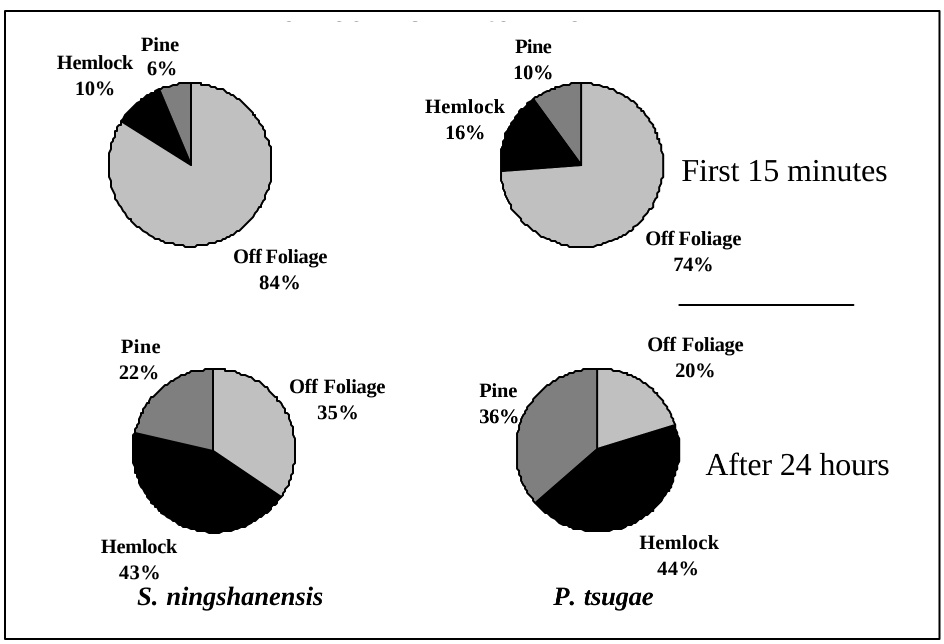

Figure 2. Location of adult lady beetles given a choice of adelgid-infested hemlock or larch foliage in a petri dish arena. Percentages are based on time spent at each location during the first 15 minutes following introduction of a beetle and the number of beetles at each location after 24 hours (N = 25).

sample t-test, Minitab 12.0), and the adults consumed nearly three-fourths of the woolly alder aphid (mean = 0.22 ± 0.10).

Table 2. Percent Prey Consumed (24 hours) in Choice Feeding Test, Hemlock Woolly Adelgid Versus Woolly Alder Aphid.

Coccinellid Species	Prey	
	HWA	WAA
Pseudoscymnus tsugae	30	7
Scymnus ningshanensis	46	7
Harmonia axyridis	51	70

In all choice tests *P. tsugae* ate significantly fewer prey than the other two lady beetle species ($P < 0.05$, two sample t-test, Minitab 12.0). This made it difficult to draw a good conclusion about the host range of *P. tsugae*.

Discussion

Host range testing can help predict the impact of biological control agents on non-targets, but Van Driesche and Bellows (1998) are critical of host range testing in the laboratory because biological control agents may be more host-limited in the environment than in the laboratory. This does not mean that host range testing in the laboratory is not beneficial or that it should not be required prior to releasing natural enemies into the environment. Careful host range testing can provide information on the specificity of biological control agents and the agents' potential for negative impacts. In this case study, *P. tsugae* and *S. ningshanensis* had narrow host ranges in the laboratory, and are predicted to have minimal impact on non-target organisms in the environment. Our tests indicated that *H. axyridis*, which is known to be a generalist, could have major ecological consequences , including negative impacts on the woolly alder aphid.

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References

Abdel-Salam, A.H., and N.F. Abdel-Baky. 2001. Life table and biological studies of Harmonia axyridis Pallas (Coleoptera: Coccinellidae) reared on the grain moth eggs of *Sitrotroga* *cerealella* Olivier (Lepidoptera: Gelechiidae). *Journal of Applied Entomology*. 125(8): 455-462.

Annand, P.N. 1924. A new species of Adelges (Hemiptera, Phylloxeridae). *Pan-Pacific Entomologist*. 1(2): 79-82

Anonymous. 1968. Adelgids (Adelges spp.) – Virginia (Weidhass, Freund). *Cooperative Extension Insect Report* 18(24):526.

APHIS. http://www.aphis.usda.gov/ppq/permits/predpar.htm. (Date accessed: 2/1/02).

Cheah, C.A.S.-J., and M. McClure. 1996. Exotic natural enemies of *Adelges tsugae* and their prospect for biological control, pp. 103-112. In Salom, S.M., T.C. Tigner, and R.C. Reardon (eds.). *First Hemlock Woolly Adelgid Review*, 12 October 1995, Charlottesville, Virginia. U.S. Department of Agriculture, Forest Service, Morgantown, West Virginia.

Cheah, C.A.S.-J. and M. McClure. 1998. Life history and development of *Pseudoscymnus tsugae* (Coleoptera: Coccinellidae), a New Predator of the Hemlock Woolly Adelgid (Homoptera: Adelgidae). *Environmental Entomology*. 27(6): 1531-1536.

Cottrell, T.E. and K.V. Yeargan. 1998. Intraguild predation between an introduced lady beetle, Harmonia axyridis (Coleoptera: Coccinellidae), and a native lady beetle, *Coleomegilla maculata* (Coleoptera: Coccinellidae). *Journal of the Kansas Entomology Society.* 71(2): 159-163.

Ferran, A., J. Gambier, S. Parent, K. Legendre, R. Tourmiere, and L. Giuge. 1997. The effect of rearing the ladybird *Harmonia axyridis* on *Ephestia kuehniella* eggs on the response of its larvae to aphid tracks. *Journal of Insect Behavior*. 10(1): 129-144.

McClure, M. S., C.A.S.-J. Cheah, and T.C. Tigner. 2000. Is *Pseudoscymnus tsugae* the solution to the hemlock woolly adelgid problem?: An early perspective, pp. 89-96. In: McManus, K.A., K.S. Shields, and D.R. Souto (eds.). *Proceedings, Symposium on Sustainable Management of Hemlock Ecosystems in Eastern North America*, 22-24 June 1999, Durham, New Hampshire. U.S. Department of Agriculture, Forest Service, Newtown Square, Pennsylvania.

Montgomery, M.E. and S.M. Lyon. 1996. Natural enemies of adelgids in North America: Their prospect for biological control of Adelges tsugae (Homoptera: Adelgidae), pp. 89-102. In: Salom, S.M., T.C. Tigner, and R.C. Reardon (eds.). *First Hemlock Woolly Adelgid Review*, 12 October 1995, Charlottesville, Virginia. U.S. Department of Agriculture, Forest Service, Morgantown, West Virginia.

Montgomery, M.E., D. Yao, and H. Wang. 2000. Chinese Coccinellidae for biological control of the hemlock woolly adelgid: Description of native habitat, pp. 97-102. *Proceedings, Symposium on Sustainable Management of Hemlock Ecosystems in Eastern North*

America, 22-24 June 1999, Durham, New Hampshire. U.S. Department of Agriculture, Forest Service, Newtown Square, Pennsylvania.

Orwig, D. A. and D. R. Foster. 1998. Forest response to introduced hemlock woolly adelgid in southern New England, USA. *Journal of the Torrey Botanical Society.* 125: 60-73.

Phoofolo, M. and J.J. Obrycki. 1998. Potential for intraguild predation and competition among predatory Coccinellidae and Chrysopidae. *Entomologia Experimentalis et Applicata.* 89 (1): 47-55.

Van Driesche, R. and T. Bellows. 1996. Biological Control, pp. 155 and 171. Natural Enemy Conservation . Chapman and Hall, New York.

Yasuda, H., T. Kikuchi, P. Kindlmann, and S. Sato. 2001. Relationships between attack and escape rates, cannibalism, and intraguild predation in larvae of two predatory ladybirds. *Journal of Insect Behavior*. 14 (3): 373-384.