Update on Rearing *Sasajiscymnus tsugae* at the Clemson Insectary and Field Monitoring of the First Release Site

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ABSTRACT

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is the primary threat to hemlock forests in eastern North America. *Sasajiscymnus tsugae* (Sasaji and McClure), an introduced coccinellid from Japan, is a biological control agent for HWA. In mass rearing *S. tsugae*, our main goals are to maximize adult beetle production, reduce the amount of human labor, and minimize production costs. Comparison studies conducted during the 2004 production year indicated that *S. tsugae* females laid significantly more eggs in oviposition jars oriented horizontally rather than vertically. An adjustment in rearing box design resulted in decreased time for watering and feeding and a mean increase of 147 adults per rearing box. Skilled personnel and attention to detail resulted in the production of 110,000 adults from an initial *S. tsugae* cohort of 200 females and 150 males. Monitoring of the first *S. tsugae* release site indicated the presence of *S. tsugae* larvae, dispersal of beetles over 130 meters from the initial release trees to other HWA-infested hemlocks, and presence of beetles at the release site over a six month period.

KEY WORDS

Biological control, mass rearing, hemlock woolly adelgid.

INTRODUCTION

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is an introduced pest that is threatening the health and sustainability of hemlock forests in the eastern United States (Knauer et al. 2002). HWA feeding can produce severe needle loss, bud death, branch dieback, and tree death in as little as four years (McClure 1995, Cheah and McClure 1998, McClure et al. 2001). HWA can cause local tree loss, regional decline, or elimination of the ecologically important eastern hemlock, *Tsuga canadensis* (L.) Carriere (Orwig et al. 2002) and Carolina hemlock, *T. caroliniana* Engelmann (McClure et al.2001).

The multi-agency HWA Steering Committee considers biological control agents to be the most environmentally and economically effective method of controlling HWA in a forest setting. Many native North American predators occasionally feed on HWA, but none have shown any significant impact on HWA populations (Wallace and Hain 2000, McClure 2001). One of the most promising non-native biological control agents against HWA is *Sasajiscymnus*

tsugae (Sasaji and McClure) (formerly *Pseudoscymnus tsugae*), a small black coccinellid from Japan (Cheah and McClure 1998).

In mass rearing *S. tsugae*, our main goals are to maximize adult beetle production, reduce the amount of human labor, and minimize production cost. One of the most important steps in mass producing *S. tsugae* under environmentally controlled conditions is accurately determining egg production. Palmer and Sheppard (2002) found that cotton gauze can be used to predict egg production. Accurate egg estimates are especially important with *S. tsugae* because larvae are cannibalistic (Blumenthal 2002); consequently, production of *S. tsugae* that reach the adult stage decreases at high larval densities. In 2004, comparison studies were conducted on egg production in oviposition jars that were oriented horizontally versus vertically. Additionally, comparative studies were conducted between conventional-style rearing boxes and modified-style rearing boxes. The first release site was monitored for the presence of *S. tsugae* reproduction, dispersal, and survivorship over a nine-month period.

METHODS AND MATERIALS

The North Carolina Department of Agriculture and Consumer Services mass rearing laboratory in Cary, North Carolina, and the Philip Alampi Beneficial Insects Rearing Lab in Trenton, New Jersey, provided the initial cohort of 350 *S. tsugae* (200 females and 150 males) for the study. Upon arrival, beetles were sexed and separated at a mean ratio of 10 females to 5 males for placement into 3.8-liter glass oviposition jars.

OVIPOSITION JARS

Based on earlier choice tests to determine the most effective gauze type for oviposition, we used Curad® basic care stretch gauze (Belersdorf, Inc., Wilton, Connecticut) for all experiments. Three 5 cm x 5 cm pieces of gauze were randomly placed among six 20-25 cm long HWA-infested hemlock twigs which were arranged into a bouquet. The hemlock bouquet was placed into an oviposition jar, a small amount of supplemental honey (as an additional food source) was spread lightly across the inside of the jar, *S. tsugae* beetles were added, and the jar's lid secured. Each oviposition jar was randomly assigned to a vertical or horizontal test group. Equal numbers of each jar orientation were setup on a daily basis (Figure 1). Oviposition jars were then placed into a controlled-environment (25 ± 1°C, 60 ± 5% humidity, and 16:8 L:D) room.

Hemlock bouquets were removed after seven days from the oviposition jars. The number of viable eggs were counted and recorded by oviposition jar orientation (vertical and horizontal). Trials were conducted from 30 November to 30 December 2004 using 300 oviposition jars. Egg production based on oviposition jar position was compared by standard analysis of variance procedures (ANOVA) (SAS Institute Inc. 2003).

REARING BOXES

Experiments to determine which rearing box style produced the highest number of adult beetles were conducted from 16 February to 6 May 2004 using 30 each conventional (Figure 2) and modified (Figure 3) rearing boxes. Modifications included use of larger wet foam blocks to maintain a more constant humidity and arrangement of hemlock twigs to minim natural orientation on trees. Each rearing box comparison contained similar numbers of eggs on gauze removed from oviposition jars on the same day. The same sequence and schedule of watering, adding HWA-infested hemlock twigs, and adding honey as a supplemental food source was used for both types of rearing boxes. The conventional and modified rearing boxes were located side by side in a controlled environment room. After 35 ± 3 days, the number of adult *S. tsugae* were collected, counted, and recorded from both styles of rearing boxes. Adult beetle production based on rearing box style was compared by standard ANOVA procedures with means separated by LSD (SAS Institute Inc. 2003).

Figure 1. Rearing jars for *Sasajiscymnus tsugae*.

Figure 2. Conventional rearing box.

Figure 3. Modified rearing box.

MONITORING

We monitored the initial release sites along a valley north of Walhalla, South Carolina, leading into the Walhalla Fish Hatchery near position 34’59"N 83’04"W. The release of 6,680 *S. tsugae* adults occurred on 12 March 2004 at a location 2460 feet above sea level. At the first location, 4,430 adults were placed on two HWA-infested hemlocks, and approximately 300 m downstream along the same hillside, 2,250 adults were placed on a single HWA-infested hemlock. The sites were monitored using visual observation to determine the number of adult beetles, larvae, eggs, or pupae in the lower canopy. The number of trees where *S. tsugae* were found and the total search time were recorded.

RESULTS AND DISCUSSION

OVIPOSITION JARS

Sasajiscymnus tsugae females laid significantly more eggs on gauze in the horizontal oviposition jars compared to those in the vertical position ($P = 0.03$) (Table 1). The horizontal oviposition jar allows the twigs in the bouquets to be arranged similarly to twigs on the hemlock trees. When running 100 oviposition jars per week, the horizontally positioned jars will weekly produce an additional 1,900 eggs on gauze per week. Based on this experiment, we now place all oviposition jars in the horizontal position.

It will be interesting to see if there is a difference in the total number of adults that are reared from eggs taken from the two jar positions. The results of this part of the experiment will not be known until the beginning of March, 2005, when this study will be completed.

REARING BOXES

The modified rearing boxes produced significantly more *S. tsugae* ($P = 0.02$) than the conventional rearing boxes (Table 2), and reduced time required for the feeding and watering process. The larger floral wet foam blocks in the modified boxes maintained a more constant moisture level. Additionally, the arrangement of the HWA-infested twigs was in an overlapping pattern along the sides of the blocks similar to how twigs naturally occur on the hemlock tree. With 100 rearing boxes, the modified boxes have the potential to produce an additional 14,700 *S. tsugae* per year. We are in the process of changing all rearing boxes to the modified arrangement to produce as many *S. tsugae* as possible for release against hemlock woolly adelgids.

MONITORING

Sasajiscymnus tsugae released above the Walhalla Fish Hatchery, Walhalla, South Carolina, provided evidence of reproduction in the field with eggs, larvae, and pupae found on HWA-infested hemlock trees at the site. Dispersal from the initial three trees to eight HWA-infested hemlocks at the site was recorded. Beetles were observed at the release site over a six-month period (Table 3). From the initial release to the first observation, there were two light snows and five days with freezing overnight temperatures at the release site.

Table 1. Comparison of *Sasajiscymnus tsugae* mean egg production ± SEM on 5 cm x 5 cm gauze strips from 30 November to 30 December 2004 at the mass rearing facility in Clemson, South Carolina, using 300 3.8-liter glass oviposition jars.

Jar Orientation	Eggs per Oviposition Jar ± SEM
Horizontal	139.2 ± 6.9a*
Vertical	120.1 ± 5.4b

*Means in a column followed by the same letter are not significantly different (LSD, P = 0.05).

Table 2. Comparison of the mean number of adult *Sasajiscymnus tsugae* ± SEM with two rearing box styles (conventional and modified) during spring 2004 at the mass rearing facility in Clemson, South Carolina, from 60 rearing boxes.

Style of Rearing Box	*Sasajiscymnus tsugae* Adults ± SEM
Conventional	434 ± (34)a*
Modified	607 ± (45)b

*Means in a column followed by the same letter are not significantly different (LSD, P = 0.05).

Table 3. Monitoring of the first release site in 2004 of *Sasajiscymnus tsugae* near the Walhalla Fish Hatchery for adults, eggs, larvae, and pupae; dispersal to other trees; and amount of time spent searching for life stages.

Date	*Sasajiscymnus tsugae*				Hemlocks with Beetles	Time
	Adults	Larvae	Eggs	Pupae		
12 March	6680	-	-	-	3	Release
24 March	120	4	-	-	3	10 min
14 April	47	2	3	-	8	2 hr
17 May	5	1	2	2	5	6 hr
16 June	3	-	-	-	2	12 hr
15 September	2	-	-	-	2	8 hr
1 December	-	-	-	-	0	8 hr

The beetles survived the cold weather and dispersed from the release trees up to 130 m to other nearby HWA-infested trees. The direction of dispersal was primarily downhill from the release trees, which could have been due to the majority of HWA-infested hemlocks being downhill from the release trees. There were indications of reproduction occurring, with observations of larvae, eggs, and pupae detected on both release trees and nearby hemlocks. At the nine-month sample date, no adult beetles were found in the lower canopy of the hemlocks. However, there was a noticeable difference in the HWA-infestation level, with release-site trees having only lightly scattered "wool" compared to heavy "wool" levels on hemlocks 200 m away. We plan to return in March 2005 to assess and compare the condition of the hemlocks at the release site to hemlocks in the next valley based on pre-release HWA-infestation data from 2004.

In the first year of mass rearing, Clemson's insectary produced over 110,000 *S. tsugae* adults and released over 100,000 beetles into the hemlock forests of South Carolina, North Carolina, and Georgia. This year's goal for the facility is to produce 150,000 *S. tsugae* for release into HWA-infested hemlocks. We plan to continue investigations on the dispersal and the long-term impacts of *S. tsugae* on HWA with studies near selected release sites.

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