Comparison of two different box styles for mass rearing of *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae), a biological control agent of hemlock woolly adelgid (Hemiptera: Adelgidae)[1]

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Abstract—The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, is the number one threat to hemlock (genus *Tsuga* Carr.; Pinaceae) forests in eastern North America. *Sasajiscymnus tsugae* (Sasaji *et* McClure), an introduced coccinellid from Japan, is a promising biological control agent for HWA. In mass rearing of *S. tsugae*, the main goals are to maximize adult beetle production, reduce the amount of human labor, and minimize production costs. Significantly higher adult *S. tsugae* production and survivorship from egg to adult were observed in a modified rearing box compared with a conventional rearing box. The 30 modified boxes produced 4400 more beetles than the 30 conventional boxes. Over time, egg to adult survivorship decreased because of larval cannibalism at densities above 1650 larvae per rearing box. Additionally, warmer weather conditions increased the number of insect and spider predators incidentally introduced via HWA-infested hemlock twigs. There was no significant difference in the time required to prepare modified and conventional boxes for initial introduction of eggs, but significantly less time was required to add water, honey, and HWA-infested twigs to the modified boxes. The additional cost and time required to add modifications to each box was compensated for by the increased number of adult *S. tsugae* produced and the time saved during the 35 days of scheduled maintenance as eggs hatched and larvae developed to adults.

Résumé—L'adelgide laineux de la pruche (HWA), *Adelges tsugae* Annand, constitue la plus grande menace aux forêts de pruches (*Tsuga* Carr.; Pinaceae) dans la zone est de l'Amérique du Nord. *Sasajiscymnus tsugae* (Sasaji *et* McClure), une coccinelle introduite du Japon, offre de bonnes possibilités pour le contrôle naturel des HWA. Dans l'élevage en masse des *S. tsugae,* on cherche avant tout à maximiser la production des coléoptères adultes et à réduire au minimum le coût de production et l'effort humain qu'il faut investir. On a constaté que la production et la survie jusqu'à l'âge adulte des coléoptères élevés dans les 30 boîtes d'élevage modifiées étaient nettement plus importantes que dans les 30 boîtes conventionnelles, soit 4400 coléoptères de plus. Avec le temps, on a constaté une baisse de l'ordre de 1650 larves par boîte dans le taux de survie à l'âge adulte, laquelle s'expliquerait surtout par le cannibalisme des larves. En plus, le climat plus chaud a eu pour conséquence de favoriser la croissance de la population d'insectes et d'araignées prédateurs dans les branches de pruches infestées par les HWA. En ce qui concerne la durée de préparation des deux types de boîtes d'élevage pour la pondaison des œufs, la différence n'était pas majeure. Par contre, on met beaucoup moins de temps à introduire de l'eau, du miel, et des branches de pruches infestées dans les boîtes modifiées. Les coût et effort supplémentaires qu'exige la modification des boîtes d'élevage sont largement compensés par le

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rendement supérieur en *S. tsugae* adultes et par les économies de temps et d'effort réalisées au cours des 35 jours d'entretien obligatoire à partir de l'éclosion des œufs jusqu'à la maturation des larves.

Introduction

The hemlock (genus *Tsuga* Carr.; Pinaceae) forests in eastern North America are being threatened by an introduced pest, the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae) (McClure 1991; Cheah and McClure 1998). HWA's preferred feeding sites are new growth and young twigs, where sap feeding causes needle discoloration and branch desiccation (Orwig and Foster 1998). HWA can cause tree death in as little as 4 years (McClure *et al.* 2001; Orwig *et al.* 2002). Both eastern hemlock, *Tsuga canadensis* (L.) Carr., and Carolina hemlock, *Tsuga caroliniana* Engelm., are susceptible to attack by HWA (Cheah and McClure 1998; McClure *et al.* 2001). Eastern hemlock's natural range in North America extends from northeastern Minnesota eastward across Wisconsin, northern Michigan, southern Ontario and Quebec, through New Brunswick, Nova Scotia, and the New England and Middle Atlantic states, westward to Indiana and Kentucky, and as far south as northern Georgia, South Carolina, and northwestern Alabama (Godman and Lancaster 1990). HWA is expanding rapidly through the hemlock forests in the eastern United States, with 15 states from Maine to Georgia reporting HWA infestations (Skinner *et al.* 2003). The adelgid's steady spread north, west, and south is causing extensive mortality and decline of hemlock forests (Evans *et al.* 1996; Knauer *et al.* 2002).

Biological control agents are the most environmentally and economically effective method of controlling HWA in a forest environment. 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). The most promising nonnative biological control agent against HWA is *Sasajiscymnus tsugae* (Sasaji *et* McClure) (formerly *Pseudoscymnus tsugae*) (Coleoptera: Coccinellidae), a small, black coccinellid from Japan (Cheah and McClure 1996). *Sasajiscymnus tsugae*'s life cycle is synchronized with HWA, and the coccinellid has demonstrated a feeding preference for adelgids. Additionally, *S. tsugae* produces multiple generations per year, adapts to a variety of climatic conditions, and possesses good searching and dispersal abilities (Cheah and McClure 1998; McClure 2001). *Sasajiscymnus tsugae* has been mass-reared as an HWA control agent in the United States since 1997 (Palmer and Sheppard 2002).

In mass rearing *S. tsugae*, the main goals are to maximize adult beetle production, reduce the amount of human labor, and minimize production costs. Observations of mass rearing techniques and methods at Ecoscientific Solutions LLC (Scranton, Pennsylvania), the North Carolina Department of Agriculture and Conservation Services mass rearing laboratory (Cary, North Carolina), and the Phillip Alampi Beneficial Insects Rearing Labs (Trenton, New Jersey) were helpful in organizing and setting up the mass rearing operation at Clemson University. Ecoscientific Solutions was observed to use a modified version of the conventional rearing box used in North Carolina and New Jersey.

Comparative studies of the two styles of rearing boxes were conducted at Clemson University's insectary to answer the following questions: Is there a difference in beetle production between the conventional and modified rearing boxes? Do initial setup and feeding times differ between rearing box styles? Are modified boxes more economical and efficient?

Materials and methods

Clemson University's starting colony of 100 female and 50 male *S. tsugae* was received from the North Carolina Department of Agriculture and Conservation Services mass rearing laboratory on 6 November 2003. The Phillip Alampi Beneficial Insects Rearing Labs provided an additional 200 *S. tsugae*, 100 on each of 17 December 2003 and 19 January 2004. Beetles from these shipments were sexed using morphological characteristics on the ventral side of the last abdominal segment and placed in glass oviposition jars at a ratio of 10 females to 5 males.

Palmer and Sheppard (2002) found that cotton gauze could be used to predict egg production, since *S. tsugae* females consistently lay nearly half of their eggs on cotton gauze. Gauze squares with known numbers of eggs were obtained from oviposition jars and used to

Fig. 1. Conventional rearing box used in mass rearing of *Sasajiscymnus tsugae* at Clemson University's insectary, Clemson, South Carolina.

Fig. 2. Modified rearing box used in mass rearing of *Sasajiscymnus tsugae* at Clemson University's insectary, Clemson, South Carolina.

compare beetle production between two different styles of rearing boxes.

The conventional clear plastic rearing box measured 61 cm × 61 cm × 49 cm. Centered 20 cm diameter holes were cut into the box's sides and top, and fine netting (625 holes per square inch) was glued over the holes. Access to the rearing box was through a 40 cm long × 20 cm diameter cloth sleeve centered within a 38 cm × 48 cm × 0.5 cm clear plastic door, which covered a 31.5 cm × 38 cm hole and was held in place with ten 2 cm × 0.5 cm diameter steel bolts and wing nuts (Fig. 1). Each conventional rearing box was initially established with six wet floral foam blocks (9.8 cm × 7.3 cm × 4.5 cm) (FloraCraft Corp., Ludington, Michigan) soaked with distilled water. A folded, white, 30.5 cm × 30.5 cm × 0.5 cm Mainstays™ washcloth (Wal-Mart Stores, Inc., Bentonville, Arkansas) was placed beneath each wet foam block to absorb excess water. Twelve HWA-infested hemlock twigs (20–25 cm long) were inserted into each wet foam block near one end. Gauze squares with a known number of *S. tsugae* eggs were randomly placed among the HWA-infested hemlock twigs (12 to 30 squares per rearing box).

The modified rearing box was constructed in the same way as the conventional box except for the addition of three 15 cm × 15 cm × 0.5 cm attachment blocks held in place with four 2 cm × 0.5 cm diameter nylon bolts with wing nuts. The bottoms of the attachment blocks were positioned 2 cm from the box's bottom, with the fronts of the three attachment blocks located 9.5 cm from the left front corner, 9.5 cm from the left side corner, and 9.5 cm from the left back corner (Fig. 2). Each attachment block had two 18 cm long × 2 cm diameter hard plastic rods sharpened to a point at the outer end. The rods were centered vertically and mounted 5 and 9 cm from the attachment block's bottom. Each rod was connected to the attachment block with a 2-cm steel screw.

A press (Fig. 3) was designed to make two holes in FloraCraft wet floral foam blocks (22.5 cm × 9.8 cm × 7.3 cm). Holes were made prior to soaking blocks in distilled water for 24 h. After soaking, the wet foam blocks were slid along the two holding rods until snug against the attachment blocks. A white 30.5 cm × 30.5 cm × 0.5 cm Mainstays™ washcloth was placed beneath each wet foam block to absorb excess water. Twenty-four HWA-infested hemlock twigs (20–25 cm long) were inserted along the bottom edge of each wet foam block. Gauze squares (12 to 30 per box) with a known number of *S. tsugae* eggs were randomly placed among the HWA-infested hemlock twigs in the three blocks of each modified rearing box.

Trials to determine which box style resulted in the highest adult beetle production were conducted from 16 February to 6 May 2004 using 30 matched pairs of rearing boxes (one conventional and one modified box). Within each pair, each rearing box contained similar numbers of *S. tsugae* 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

Fig. 3. Press used to make holes in wet foam blocks placed in modified rearing boxes at Clemson University's insectary, Clemson, South Carolina.

supplemental food source was used for both types of rearing boxes. Matched pairs of rearing boxes were located side by side in the same controlled environment (25 ± 1 °C, 60 ± 5% RH, and 16L:8D). After 35 ± 3 days, adult *S. tsugae* were collected from both styles of rearing boxes and counted.

Production of adult *S. tsugae* beetles and survivorship from egg to adult were compared between the two rearing box styles by standard analysis of variance procedures with means separated by LSD (SAS Institute Inc. 1997). Percent survivorship from egg to adult by type of rearing box was plotted from 16 February (week 15) to 6 May (week 26). No comparisons were conducted on week 19 while additional modified rearing boxes were being constructed. The mean numbers of eggs per rearing box per week were plotted from 16 February (week 15) to 6 May (week 26).

The amount of time required for technicians to prepare each rearing box style (establishment of wet foam blocks and insertion of the initial HWA-infested hemlock twigs) and the amount of time spent watering the wet foam blocks, adding additional HWA-infested hemlock twigs, and adding honey were compared between the conventional and modified rearing boxes using standard analysis of variance procedures with means separated by LSD when appropriate (SAS Institute Inc. 1997).

Results

Significantly more adult *S. tsugae* were obtained from the modified rearing boxes than from the conventional boxes (Table 1). A total of 17 177 beetles were reared from 30 modified boxes, whereas 12 775 beetles were reared from 30 conventional boxes. Survivorship from egg to adult was significantly higher in the modified rearing boxes than in the conventional rearing boxes (Table 1). Overall, there was no significant difference ($F_{2,59} = 0.012$, $P = 0.91$) in the mean (±SEM) number of introduced *S. tsugae* eggs per box, with 1733 ± 66 eggs placed in modified boxes and 1722 ± 67 eggs placed in conventional boxes.

Survivorship from egg to adult in the modified rearing boxes decreased from 67% in mid-February to 17% in early May (Fig. 4). Survivorship from egg to adult in the conventional rearing boxes decreased in an almost linear fashion from 44% in mid-February to 9% in early May (Fig. 4). Higher survivorship was observed in the modified rearing boxes than in the conventional rearing boxes. Survivorship dropped below 4% from the last week of May (week 29) until the end of the rearing season on 14 July 2004 (week 36) (data not shown).

The weekly mean number of eggs placed in each rearing box increased from 1270 in week 15 to a maximum of 2045 in week 20 (Fig. 5). The mean number of eggs per rearing box remained high until dropping to 1100 on week 26 (Fig. 5). There was a slight increase in survivorship in weeks 23 and 26, when the mean number of eggs per box dropped below 1650 (Figs. 4, 5).

There was no significant difference in the amount of time required to prepare rearing boxes, but the conventional box required slightly more time to prepare initially (Table 2). For scheduled box maintenance, significantly more time was required to add HWA-infested twigs, water, and honey to the conventional box compared with the modified box (Table 2).

The additional cost of the box modification was $45.00 per box. Installation of box modifications required 20 min per box.

Table 1. Comparison of the mean (±SEM) number of adult *Sasajiscymnus tsugae* and the mean (±SEM) survivorship from egg to adult between two rearing box styles from 16 February to 6 May 2004 at Clemson University's insectary, Clemson, South Carolina.

Rearing box style	No. of adults reared per box	Survivorship to adult (%)
Conventional	434±34*a*	24.6±2.1*a*
Modified	607±45*b*	34.4±3.0*b*

Note: The blocking variable was start date for each set of paired cages. Within a column, means followed by the same letter are not significantly different (LSD; P = 0.003 for no. of adults, P = 0.004 for survivorship).

Fig. 4. Comparison of mean weekly survivorship of *Sasajiscymnus tsugae* from egg to adult by rearing box style from 16 February to 6 May 2004 at Clemson University's insectary, Clemson, South Carolina. Week 19 is excluded because additional modified boxes were being constructed.

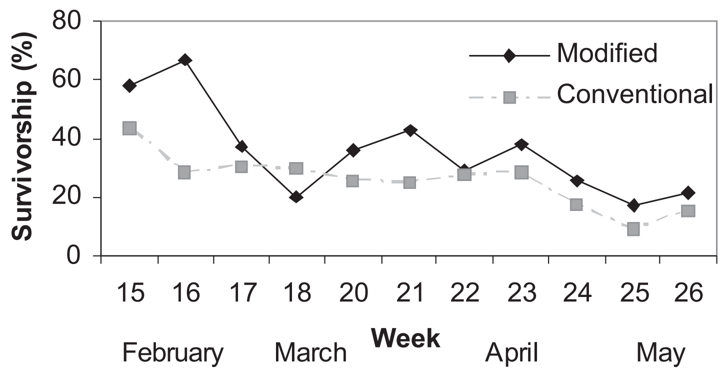
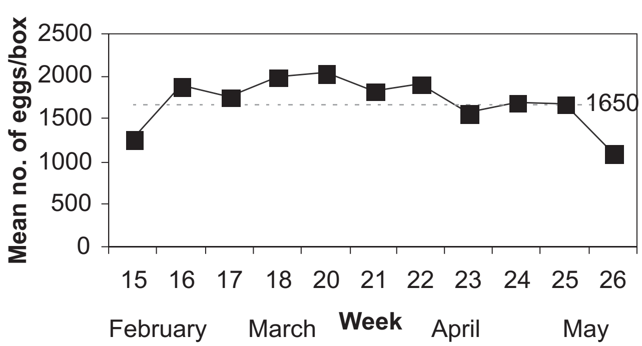


Fig. 5. Mean number of *Sasajiscymnus tsugae* eggs per rearing box from 16 February to 6 May 2004 at Clemson University's insectary, Clemson, South Carolina. Week 19 is excluded because additional modified boxes were being constructed.



Discussion

In mass rearing *S. tsugae*, the main goals are to maximize adult beetle production under environmentally controlled conditions, reduce the amount of human labor, and minimize production costs. Our experiments indicate that the style of rearing box does make a difference in adult beetle production. *Sasajiscymnus tsugae* survivorship from egg to adult and overall adult production were significantly higher in the modified rearing boxes than in the conventional rearing boxes. The larger wet foam blocks in the modified rearing boxes maintained moisture levels better and kept the hemlock twigs greener for a longer period of time than the smaller blocks in the conventional rearing boxes.

Survivorship in both conventional and modified rearing boxes decreased through time in

Table 2. Comparisons of mean (±SEM) time required for technicians to set up two different styles of rearing boxes for *Sasajiscymnus tsugae* and to add HWA-infested twigs, water, and honey to the boxes at Clemson University's insectary, Clemson, South Carolina.

Rearing box style	Time to set up box (s)	Time to add twigs, water, and honey (s)
Conventional	433±22*a*	228±15*b*
Modified	371±15*a*	180±13*a*

Note: Within a column, means followed by the same letter are not significantly different (LSD, $P = 0.03$).

association with cannibalism by *S. tsugae* larvae, inadvertent addition of insect and spider predators to rearing boxes via HWA-infested hemlock stems, and a decrease in the number of HWA eggs and crawlers near the onset of summer diapause. When the larval density was above 1650 per box, there was a decrease in survivorship, partially due to cannibalism. Blumenthal (2002) reported larval cannibalism in sleeve cage studies in Pennsylvania. Other potential causes of decreased survivorship were predatory insects and spiders that were incidentally introduced into the rearing boxes on HWA-infested twigs. From the insides of rearing boxes, we removed neuropterans (Chrysopidae, Coniopterigidae, and two species of Hemerobiidae), coleopterans (Derodontidae and Coccinellidae (*Harmonia axyridis* Pallas)), dipterans (Syrphidae and Chamaemyiidae (*Leucopis* (*Leucopis*) *piniperda* Malloch)), hemipterans (Pentatomidae, Miridae, Rhyparochromidae, and Nabidae), and five spider families (Tetragnathidae, Araneidae, Thomisidae, Theridiidae, and Salticidae). Ovtsharenko and Tanasevitch (2002) collected spiders at Black Rock Forest, a 1530-ha scientific reserve in Orange Co., New York, at all levels of the hemlock forest: from soil and leaf litter; under the bark of hemlock trees; and on trunks, branches, twigs, and leaves, including those at the tops of small hemlock trees. They identified 109 species of spiders from 15 families with associations to hemlock forests. Additionally, 29 spider species from 10 families were found on hemlock branches, twigs, and leaves.

By early June, the number of HWA eggs and crawlers declined in association with the onset of summer aestivation. Additionally, there seems to have been a decrease in food quality with the sisten eggs laid by the progrediens in late May and June. Sisten crawlers settle on young hemlock branches, begin to feed, and then enter an aestival diapause that lasts through the summer months (McClure *et al.* 2001). In the rearing boxes, survivorship from egg to adult for *S. tsugae* dropped below 4% in early June and then decreased until the end of the rearing season in association with the onset of settlement of adelgids for aestivation.

Preparing a modified rearing box for *S. tsugae* eggs requires slightly less time than preparing a conventional rearing box. Maintenance (watering wet foam blocks, adding honey, and adding twigs) of the modified rearing boxes requires significantly less time than maintenance of the conventional rearing boxes. This overall time saving per rearing box is significant because each rearing box receives water 10 times between initial setup and collection of *S. tsugae* adults. Based on the maintenance schedule for each box, saving 48 s per rearing box results in a minimum time savings of 144 min per week when all 90 rearing boxes are in production, with an estimated cost savings of $15 000 per year.

Conventional rearing boxes at Clemson University are being modified for next season's *S. tsugae* production. The additional cost for modifications will be offset by the increase in *S. tsugae* adult production and the time savings gained during rearing box maintenance.

Sasajiscymnus tsugae has been released in 11 states from New Hampshire to South Carolina and is considered to be established in 7 states (McClure 2001). It has two generations per year in the field in colder states such as Connecticut, where the adults overwinter on hemlock foliage during mild winters (McClure 2001; Montgomery *et al.* 2003). In Connecticut, *S. tsugae* adults have been documented dispersing over 0.5 miles from a release site (McClure 2001). The small coccinellids have helped remove 47% to 88% of woolly adelgids infesting hemlock forests at study sites in Virginia and Connecticut (McClure and Cheah 1998). In the first year of mass rearing, Clemson University's insectary produced over 110 000 *S. tsugae* adults, and we released over 100 000 beetles in the hemlock forests of South Carolina, North Carolina, and Georgia. Next year, we plan to increase the number of adult *S. tsugae* produced, partially through modifications to rearing boxes. Dispersal and long-term impacts will be evaluated in studies near selected release sites. Additionally, another promising HWA biological control agent, *Laricobius nigrinus* Fender, which is native to the Pacific Northwest (Zilahi-Balogh *et al.* 2003), will be mass produced at Clemson University's insectary beginning in the fall of 2004.

Additional research is needed to help answer questions that arose during the mass rearing of *S. tsugae.* Is there a difference in egg production using eastern hemlock *versus* Carolina hemlock? Are there differences in HWA and *S. tsugae* biology between the southern Appalachians and the northern Appalachians?

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