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SHORT COMMUNICATION

Recovery of *Sasajiscymnus tsugae*, released against hemlock woolly adelgid, *Adelges tsugae,* in the southern Appalachians

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Eastern hemlock in the Great Smoky Mountains National Park is currently threatened by the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). As part of a management plan against this invasive insect pest, about 350,000 adults of the predatory beetle *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae) were released at ca. 150 sites in the Park from 2002 to 2007. Of these adult release sites, 33 were sampled in 2008 and 2009 using beat-sheet sampling for 4 man-hours. *Sasajiscymnus tsugae* adults ($n = 78$) and/or larvae ($n = 145$) were recovered from seven sites (21.2% of the release sites sampled). Recovery of *S. tsugae* was significantly associated with older release sites, with the most beetles recovered from 2002 release sites. These results indicate that *S. tsugae* may require more time (i.e., 5–7 years) than anticipated for population densities to reach readily detectable levels in some areas.

Keywords: *Sasajiscymnus tsugae*; biological control; *Adelges tsugae*; predator recovery; Great Smoky Mountains National Park; *Tsuga canadensis*

Populations of eastern hemlock, *Tsuga canadensis* (L.) Carrière, have experienced widespread mortality throughout the eastern United States because of excessive feeding by hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae) (Vose et al. 2008). Since its introduction from Asia, HWA has gradually spread into 17 states in the eastern United States (Trotter, Courter, Turcotte, and Onken 2008), and since it was first documented in the Great Smoky Mountains National Park in 2002, thousands of eastern hemlocks have died (JFG, Personal observation).

As part of a classical biological control program to introduce a complex of natural enemies against HWA, *Sasajiscymnus tsugae* (Sasaji and McClure) (Coleoptera: Coccinellidae), which is native to Japan, was the first introduced species of natural enemy selected for mass rearing and release against HWA in the United States beginning in Connecticut in 1995 (Cheah, Mayer, Palmer, Scudder, and Chianese 2005). Since 2002, more than two million *S. tsugae* beetles have been reared

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in insectaries in several states and released throughout the eastern United States, with many of the releases made in the southeastern United States (Grant 2008; Salom, Kok, Lamb, Jubb, and Onken 2008). Of these, approximately 350,000 were released at ca. 150 sites in the Great Smoky Mountains National Park from 2002 to 2007. However, limited information exists on the establishment of these introduced natural enemies in areas of the releases in the eastern or southeastern United States (McClure and Cheah 1998; Cheah and McClure 2000; Grant 2008; McDonald et al. 2008).

In 2008, a study was initiated to evaluate the establishment of *S. tsugae* released against HWA in the Great Smoky Mountains National Park. The objective of this study was to determine the presence and extent of established populations of this introduced predatory species at multiple release sites in the National Park.

Sampling was conducted in 2008 and 2009 in the Great Smoky Mountains National Park, which is located in the southern Appalachians spanning the border of North Carolina and Tennessee. Eastern hemlock trees at each release site were sampled using beat sheets for *S. tsugae*. Coordinates for all *S. tsugae* release sites were obtained from Park personnel, and sites were located using a Garmin® GPS map 60 CSx6 GPS unit. Original release trees, which were identified by an aluminum tag, at each site were sampled where possible. Each site was sampled once except Laurel Falls 1, Laurel Falls 2, Laurel Falls 3 and Anthony Creek; each of these four sites was sampled in both years. Sampling was conducted from 5 May to 7 July 2008 and 25 February to 16 June 2009 (this period coincided with expected activity of *S. tsugae*). At each site, beat-sheet sampling was conducted for 4 man-hours. On all hemlock trees in the release site area, accessible branches from ground level to a height of 2.5 m were sampled by tapping them five to eight times with a wooden rod while holding a white beat sheet (71×71 cm) beneath the branch to catch dislodged predators. Depending on the size of the tree, one to three beat-sheet samples were collected
per tree.

All *S. tsugae* observed during beat-sheet sampling were recorded, and representative specimens of suspected adults and larvae of *S. tsugae* were taken to the laboratory, where larvae were reared in glass jars (2.64 L) on HWA-infested hemlock until pupation and emergence as adults. Field-collected and laboratory-reared adults were identified in the laboratory (GJW), and species identification was confirmed by James Parkman at the Lindsay Young Beneficial Insects Laboratory (University of Tennessee). Voucher specimens are housed in the Integrated Pest Management and Biological Control Laboratory at the University of Tennessee.

The number of larvae, adults, and both stages of *S. tsugae* recovered from all sites sampled were compared among years using a Chi-square (χ^2) test. Pearson's correlation was performed to qualify the relationship (i.e., positive or negative) between year of release and number of larvae, adults, and both stages of *S. tsugae* recovered. All alpha levels were $P \leq 0.05$, and analyses were conducted using the statistical package SPSS 17.0 (SPSS, Chicago, IL, USA).

Thirty-three *S. tsugae* release sites (ca. 3,500 trees) were sampled in 2008 and 2009 (Figure 1). These sites represented releases made in 2002 (nine sites), 2003 (one site), 2004 (four sites), 2005 (three sites), 2006 (10 sites), and 2007 (six sites). During this study, *S. tsugae* adults ($n = 78$) and/or larvae ($n = 145$) were recovered from seven (21.2%) of these release sites (Figure 1, Table 1). The greatest numbers of adult

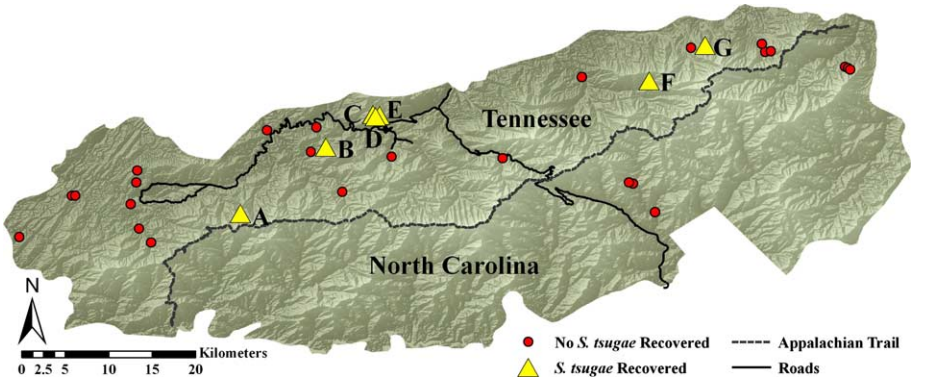

Figure 1. Release ($n = 33$ sites) and recovery ($n =$ seven sites) of *Sasajiscymnus tsugae* from eastern hemlock in the Great Smoky Mountains National Park, 2008–2009 (A, Anthony Creek; B, Buckthorn Gap; C, Laurel Falls 1; D, Laurel Falls 2; E, Laurel Falls 3; F, Ramsey Cascades; and G, Buckeye Creek) (symbols indicate sites where *S. tsugae* have been released and sites where *S. tsugae* have been recovered).

S. tsugae were recorded from Buckthorn Gap ($n = 22$) (Site B) and Laurel Falls 2 ($n = 15$; sampled 2009) (Site D) while only one *S. tsugae* was recovered from Ramsey Cascades (Site F) (Figure 1, Table 1). The greatest numbers of larvae were recovered from Laurel Falls 2 ($n = 83$; sampled 2008) (Site D), Laurel Falls 1 ($n = 40$; sampled 2008) (Site C), and Buckthorn Gap ($n = 18$) (Site B) (Figure 1, Table 1).

Table 1. Recovery of *Sasajiscymnus tsugae* (St) on eastern hemlock, *Tsuga canadensis*, at seven of 33 release sites in the Great Smoky Mountains National Park (recoveries were documented in 2008–2009).*

Location	Date of release	Date recovered	No. St beetles released	No. St adults recovered	No. St larvae recovered
Anthony Creek (A)**	26 Jun 2002	13 Jun 2008	2,923	0	1
		23 Apr 2009		13	0
				6.5/site	0.5/site
Buckthorn Gap (B)	25 Jun 2002	21 May 2009	3,118	22	18
Laurel Falls 1 (C)	03 Jun 2002	11 Jun 2008	2,039	0	40
		8 Jun 2009		13	0
				6.5/site	20.0/site
Laurel Falls 2 (D)	3 Jun 2002	11 Jun 2008	1,940	0	83
		8 Jun 2009		15	2
				7.5/site	42.5/site
Laurel Falls 3 (E)	10 Jul 2002	8 Jun 2009	848	12	1
Ramsey Cascades (F)	3 May 2007	10 Mar 2009	2,386	1	0
Buckeye Creek (G)	22 Feb 2006	4 May 2009	2,045	2	0
Total				78	145

**Sasajiscymnus tsugae* was recovered at seven of 33 release sites; only information for the seven release sites where *S. tsugae* was recovered is provided. The 33 release sites represented releases made in 2002 (nine sites), 2003 (one site), 2004 (four sites), 2005 (three sites), 2006 (10 sites), and 2007 (six sites).
**Letters in parentheses correspond to locations on the release and recovery map shown in Figure. 1.

The percentages of release sites that were positive for the recovery of *S. tsugae* was influenced by the year of the release. *Sasajiscymnus tsugae* was recovered from 55.5% (5 of 9) of the 2002 release sites, but recovered from only 10.0% (1 of 10) and 16.6% (1 of 6) of the 2006 and 2007 release sites, respectively (Table 1). Adult *S. tsugae* were recorded from 2002 ($n = 75$), 2006 ($n = 2$), and 2007 ($n = 1$) release sites. Larvae of *S. tsugae* were found at only 2002 ($n = 145$) release sites. These results indicate that significantly greater numbers of larvae ($\chi^2 = 16.892$, df = 1, $P < 0.001$), adults ($\chi^2 = 16.676$, df = 1, $P = 0.005$), and total ($\chi^2 = 25.973$, df = 1, $P < 0.001$) *S. tsugae* were recovered from sites where releases were made in 2002 than all other sites sampled. Additionally, a significant inverse relationship between year of release and number of larvae (Pearson = -0.316; $P = 0.029$), adults (Pearson = -0.447; $P = 0.003$), and total (Pearson = -0.440; $P = 0.003$) *S. tsugae* was documented.

Previous efforts to recover and/or document establishment of *S. tsugae* on HWA have yielded mixed results. For example, low numbers of *S. tsugae* were recovered three months after their initial releases in the Great Smoky Mountains National Park in 2002 from 3 of 10 sites. These sites included Laurel Falls 2 ($n = 2$ adults) (Site D), Laurel Falls 3 ($n = 1$ adult) (Site E), and Anthony Creek ($n = 1$ adult) (Site A) (Figure 1) (Buck, Lambdin, Paulsen, Grant, and Saxton 2005; Lambdin, Grant, Paulsen, and Saxton 2006). During 2003 (one year after these initial releases), *S. tsugae* was not recovered from any of the 10 release sites (Lambdin et al. 2006). In the current study, however, all three of these release sites were documented as positive recovery sites in 2008 and/or 2009. In similar studies on hemlock in Connecticut and North Carolina, *S. tsugae* were recovered 1–6 years after their initial releases (Cheah and McClure 2000; Cheah et al. 2005; McDonald et al. 2008). In Georgia, however, no *S. tsugae* were recovered from six sites two years following releases (M. Dalusky, personal communication).

Despite the seemingly large numbers (more than 350,000) of beetles that have been released in the National Park (ca. 210,000 ha) since 2002, this number equivocated to only 1.8 *S. tsugae*/ha in the Park (Grant 2008). If only the area of hemlock-dominated forest (ca. 36,000 ha) is considered (GRSM 2010), *S. tsugae* has been released at a density of 10.8 beetles/ha. In many biological control programs, predators are released at much greater densities, usually to attain a rapid reduction in pest populations. As compared to *S. tsugae*, HWA can increase its population rapidly. For example, the parthenogenic HWA undergoes two generations each year with about 300 and 50 progeny per female for the first and second generations, respectively (all progeny are female). On the other hand, *S. tsugae* undergoes one generation each year under field conditions with about 100–200 progeny (males and females) per female. These reproductive and generational differences, combined with the relatively low numbers released per hectare, partially explain the slow increase in predator populations.

Sampling efforts during this study were restricted by necessity to the canopy closest to the ground (within 3 m). However, many of the hemlocks sampled were between 5 and 20 m tall, and some hemlocks were more than 30 m tall. *Sasajiscymnus tsugae* has been found in the upper canopy shortly after release (Cheah et al. 2005), and heavily-infested hemlocks provide ample prey material throughout the canopy. Therefore, the detection of low numbers of *S. tsugae* when sampling a fraction of the potential habitat on a single tree is promising. If *S. tsugae* were detected in the lower one-third to one-fourth of the canopy and larger numbers

are expected in the upper canopy, then the established population of *S. tsugae* in the National Park is greater than that estimated in our research. However, recovery of predatory beetles in release sites in the National Park does not necessarily indicate improved tree health. Although beetles have established in several areas of the Park, their populations may not have increased sufficiently to reduce infestations of HWA to non-damaging levels.

This ongoing study documents the presence of *S. tsugae* persisting at seven locations in the Great Smoky Mountains National Park. Populations of *S. tsugae* are well established (present 6+ years after release and adults and larvae recovered) at five of these sites. Results indicate that *S. tsugae* may take longer (as many as 5–7 years) than anticipated for populations to establish and attain readily measurable levels, as relatively greater numbers of *S. tsugae* were recovered from releases made in 2002 compared to releases made in subsequent years (Table 1). Although *S. tsugae* was the first introduced predator species to be released en masse against HWA, it is one of several introduced predators, including species in the genera *Laricobius*, *Scymnus* and others, that have been released, or are being considered for release, as part of a natural enemy complex (Asaro, Berisford, Montgomery, Rhea, and Hanula 2005; Salom et al. 2005). Therefore, establishment of *S. tsugae* on HWA can only enhance future efforts involving the releases of new biological control agents.

Additional release sites will be sampled to fully assess establishment of *S. tsugae* in the Great Smoky Mountains National Park and investigate the conditions which may contribute to its establishment. Concurrently, tree health and HWA population data will be obtained by Park personnel and others. These findings will be combined and analyzed to assess the impact of *S. tsugae* on HWA and to develop a predictive model to help to understand factors that influence establishment of *S. tsugae* on eastern hemlock.

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