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# Detection of Hemlock Woolly Adelgid (Hemiptera: Adelgidae) Infestations with Sticky Traps

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## Abstract

We deployed sticky traps underneath the crown of eastern hemlock, *Tsuga canadensis* (L.) Carrière, to assess their sensitivity at detecting crawlers (1st instar nymphs) of the non-native hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). We found these traps more sensitive at detecting infested trees with low densities of *A. tsugae* than branch-tip sampling with pole pruners. We observed two peaks of crawler abundance at all sites: these peaks likely represented the timing of the progrediens and sistens crawler stages of *A. tsugae*. Deployment of sticky traps in treated and high-risk stands may prove useful at detecting residual and new infestations, respectively.

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The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a bivoltine insect with a polymorphic (sistens, progrediens, and sexuales) life cycle that is obligatorily parthenogenetic in its invaded range (McClure 1989, Fitzpatrick et al. 2012). Each morph has 3 stages of development: egg, nymph (4 instars), and adult (McClure 1989). Crawlers are first instar nymphs and the only stage that is mobile. Crawlers become sessile as soon as they insert their mouthparts into tree tissues to feed (McClure 1989). In early spring, before bud burst in eastern hemlock, *Tsuga canadensis* (L.) Carrière, progrediens crawlers emerge from sistens ovisacs and attempt to settle on the stem at the base of a hemlock needle from the previous year's growth (hereafter twig) amongst their sistens mothers. After bud burst, progrediens adults lay sistens eggs. Sistens crawlers emerge and settle on the stem at the base of a hemlock needle on either the current or previous year's growth. First instar sistens crawlers undergo an aestival diapause and develop throughout fall and winter, maturing in spring (McClure 1989). Natural dispersal of crawlers is facilitated by wind, rain, and animal activity (McClure 1990), but many crawlers become dislodged and likely land on non-host plants and the forest floor near their point of origin (McClure 1989, Turner et al. 2011).

Populations of *A. tsugae* presently found in the eastern United States originated from southern Japan (Stoetzel 2002, Havill et al. 2006). From its original detection in Virginia (Richmond) in 1951, *A. tsugae* has spread to 20 other states and the District of Columbia and has likely killed millions of *T. canadensis* (e.g., Orwig et al. 2002). The loss of stands dominant in hemlock is tragic because such stands create unique conditions within which several animal species flourish (Ellison et al. 2005, Adkins and Rieske 2013, Orwig et al. 2013). The adelgid can now be found in states as far north as New York, Vermont, New Hampshire, Maine, and occasionally as far northwest as Michigan (USDAFS 2014). These states all share a border with eastern Canadian provinces. In Canada, A. tsugae is a regulated pest (CFIA 2014a) and as such is the subject of

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a directive (D-07-05) or quarantine program designed to prevent its introduction and spread (CFIA 2014b). Its presence in Canada could lead to economic impacts and to an ecological loss; however, loss of hemlock in riparian environments is particularly concerning (Brantley et al. 2013). Eastern hemlocks infested by *A. tsugae* were detected recently in eastern Canada at two locations in southern Ontario (NAPPO 2014) using a ground-based visual assessment of lower crown branches modified from Costa and Onken (2006). To prevent further spread of this insect in eastern Canada, the Canadian Food Inspection Agency (CFIA) removed within weeks of detection all trees known to be infested at the time and incinerated all woody material (L. Shields, CFIA, personal communication). At both locations, more infested trees were found the following year (NAPPO 2014); these trees were also destroyed within weeks of detection.

The canopy distribution of *A. tsugae* at the early stage of an invasion is unknown, but the upper crown may be the first to become infested (Evans and Gregoire 2007). Sampling the upper crown of mature hemlock is time consuming due to the height of branches (> 15 m) above ground; in many instances, this foliage is out of reach of conventional, relatively rapid assessment techniques, such as pole pruning and visual assessment. There is an urgent need to develop early detection tools that permit evaluation of all possible hemlock habitats rather than just a portion of it, particularly if eradication continues to be the response to new finds.

Alternatively, if a trap could be used to detect dispersing 1st instar *A. tsugae* crawlers during the earliest stages of *A. tsugae* establishment, then directly sampling the upper crown of hemlock trees is not necessary. We investigated the efficacy of sticky traps deployed under the canopy of individual trees at detecting the presence of *A. tsugae* crawlers in hemlock stands and compared it to sampling branch tips from the lower half of the crown with pole pruners. As an *a posteriori* objective, we looked at the relationship between the abundance of ovisacs on branch tips per tree to that of crawlers on traps under the same trees.

## Materials and Methods

**Study Sites.**  We carried out this study at three sites in upper New York State from May to July 2014: Texas Hollow (TH) lat. 42.416031°N, long. 76.794492°W; Rattlesnake Hollow (RH) lat. 42.541684°N, long. 76.852352°W; and Mill Creek (MC) lat. 42.585108°N, long. 76.816722°W. The trees at TH were the largest used in this study (mean height = 27.7 ± 1.0 m; mean height to live crown = 7.0 ± 1.4 m; mean DBH = 47.4 ± 2.4 cm) followed by those at MC (21.2 ± 1.2 m; 3.8 ± 1.1 m; 39.8 ± 1.9 cm) and RH (18.7 ± 1.3 m; 1.2 ± 0.1 m; 42.0 ± 2.3 cm).

**Sticky Traps.**  We purchased pairs of commercially available green prism traps (Synergy Semiochemicals Corp., Burnaby, BC, Canada). Each pair comes with one side already coated with a clear insect trapping glue (facing each other). We cut 6 squares (25 × 25 cm; 625 cm²), two from each panel of the pair of prism traps. We selected six trees at least 20 m apart at each site and installed two sticky traps (sticky side up) under each one. We nailed each trap to the top end of a wooden stake (2.5 × 2.5 × 200 cm) hammered about 70 cm deep into the ground. One trap was placed within 1 m of the bole (interior) and the other was placed at the outer edge of the drip line (exterior) on the same side of the tree; inter-trap spacing under a tree varied between 3 and 5 m. We set up the first traps on 5 May before progrediens crawlers had emerged.

We replaced traps every 2 wk until 10 July at MC and RH and 31 July at TH, after most sistens crawlers had settled on branches. We placed each used-up trap into a clear 7.6 L Ziplock® Hefty Jumbo Slider bag (S.C. Johnson & Son Inc., Racine, WI) and stored the bags at -15°C until we could examine the trap with a dissecting microscope (64×) (Fig. 1). To assess crawler abundance, we left the traps in Ziplock bags. We drew two sets of lines on each bag. The
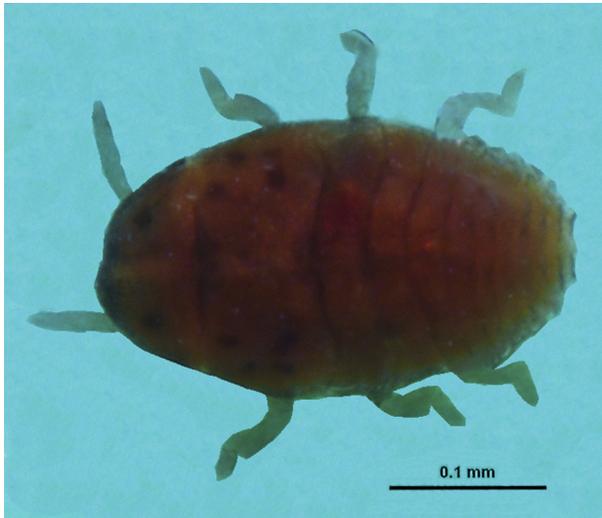
Figure 1. Example of first instar crawler of *A. tsugae* caught on green sticky trap. Image was created using the extended depth of focus (EDF) module of Nikon NIS-Elements v. 4.0.08 software and touched up using Adobe Photoshop CS6 v. 13.0. Photo: Gene Jones.

first set consisted of two perpendicular lines passing through the centre of the trap, resulting in four quadrants: $12.5 \times 12.5$ cm. We subdivided further each quadrant by tracing four lines parallel to the trap's edge at 2 cm intervals starting at 2 cm from the edge of the trap. This second set of lines created four rectangles of $2 \times 12.5$ cm in each quadrant for a total of 100 cm² per quadrant. Thus, when crawler abundance was low (running total per quadrant < 100), we examined a maximum of 400 out of 625 cm² per trap and recorded the number of crawlers. When abundance was high (cumulative total per quadrant ≥ 100), we calculated crawler density using the following formula: running crawler count ÷ area scanned in cm² × 10,000 (to convert cm² to m²). Because the duration of collection periods sometimes varied by 2 to 3 days amongst sites, we standardized estimates of crawler interception rate by dividing crawler density by the number of days traps were in the field.

**Branch-Tip Sampling.** We estimated *A. tsugae* populations for each of the six trees paired with a set of sticky traps. On 5–6 May (sistens ovisacs) and 9–10 July (progrediens ovisacs), we removed five 30-cm-long branch tips haphazardly from the lower half of the live crown of each tree using a modified Gilmour Commercial Tree Pruner (Robert Bosch Tool Corp., Peoria, IL; maximum reach: 8.5 m). We examined each branch tip and counted live sistens, progrediens, or dead adults with egg masses (hereafter ovisacs) found on the twigs. We also noted the length (cm) of each twig. Because densities of *A. tsugae* were low, we expressed abundance as the average number of ovisacs per 10 cm of twig length examined.

**Analysis.** We used a one-way repeated measures analysis of variance (AOV function, R Core Team 2014) to determine if a significant proportion of the variation in daily crawler interception rate was attributable to trap position (interior vs. exterior) at TH. We used simple linear regression to determine if peak interception of sistens crawlers on traps was related to abundance of sistens ovisacs in the crown, separately for each site (LM function, STATS package, R Core Team 2014). Analyses were considered significant at $P \leq 0.05$.

## Results and Discussion

Abundance of ovisacs varied from 0 to 4.5 per 10 cm of twig length. Population levels were low at all sites: mean abundance (± SE) of sistens ovisacs per 10 cm of twig length was lowest at RH (0.11 ± 0.05) < MC (0.86 ± 0.67) < TH (2.02 ± 0.71). Mean abundance of progrediens ovisacs per 10 cm of twig length was lowest at MC (0.17 ± 0.10) < RH (0.41 ± 0.19) < TH (0.53 ± 0.23).

Daily interception rates of crawlers on sticky traps varied from 0 to 1,120 crawlers per m$^2$. DNA extraction was not possible from specimens embedded in sticky coating (E. Maw, Agriculture Canada, personal communication). However, gross morphology of crawler samples coupled with use of traps under hemlocks suggests that crawlers were *A. tsugae* (Annand 1924). We noted two peaks of crawler abundance at all sites (Fig. 2). We hypothesize these peaks represented peak abundance of progrediens and sistens crawler stages, respectively. At TH, where daily average interception rate was highest amongst traps (468 ± 97 crawlers per m$^2$), variation in interception of crawlers was not explained by trap position ($F = 1.95$; df = 1, 33; $P = 0.30$).

Our data also suggest that the use of intercept traps might be more effective than branch-tip sampling in indicating the presence of *A. tsugae*-infested trees. At MC, four of the 6 trees were estimated to have '0' sistens ovisacs by branch-tip sampling; yet, traps placed under these trees intercepted from 2 to 6 crawlers per m$^2$ daily. Also, at MC, three of the six trees were estimated
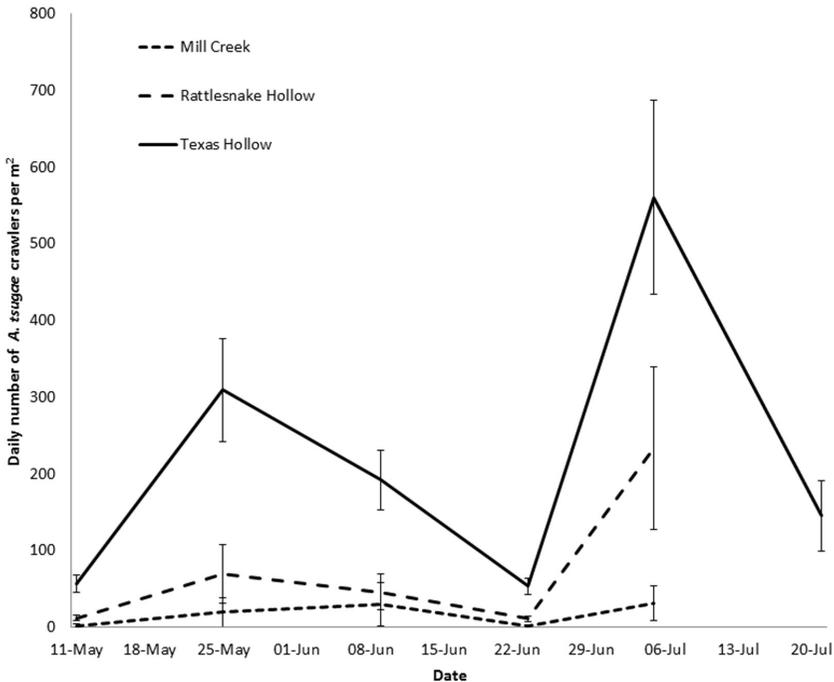


Figure 2. Daily interception rate (mean ± SE) of crawlers on green sticky traps during May to July 2014 at three sites in upper New York State. Data are presented at the midpoint dates between collections of traps. Traps were set up 5-6 May, before crawler emergence and taken down 10 July at Mill Creek and Rattlesnake Hollow and 31 July at Texas Hollow after most crawlers had settled on branches.

to have had '0' progrediens ovisacs after branch-tip sampling; yet, from 2 to 9 sistens crawlers per m$^2$ were intercepted daily under the trees. Whether crawlers intercepted on sticky traps under a tree are from that tree cannot be ascertained; however, the stand at MC consisted predominantly of mature trees scattered singly or in small groups of 2 or 3 trees and surrounded by broadleaf trees. Results from Turner et al. (2011) suggested that most dispersal events of *A. tsugae* crawlers likely occur within 25 m of the source.

Our results on the efficacy of sticky traps at intercepting crawlers are consistent with the pioneering work of McClure (1990) who used this trapping method to demonstrate the role wind played in the spread of *A. tsugae*. To our knowledge, our study is the first to use sticky traps to show the timing of the progrediens and sistens crawler activity periods of *A. tsugae*. This information may be important for the development of best management practices for *A. tsugae* or for research purposes.

Overall, the abundance of ovisacs in the crown of a tree appeared positively related to interception rate on traps (Fig. 3). However, we suspect that differences in the slopes of the regressions were due to varying abundance of hemlock and *A. tsugae* among sites. For example, the regression of ovisac abundance and peak crawler interception was strong at MC ($r^2 = 0.92$; df = 1, 5; $P = 0.001$) probably because that site consisted of relatively isolated mature hemlocks with light infestation by *A. tsugae* within a hardwood stand. There was no significant regression at either of the other two sites (TH, $P = 0.37$; RH, $P = 0.44$).
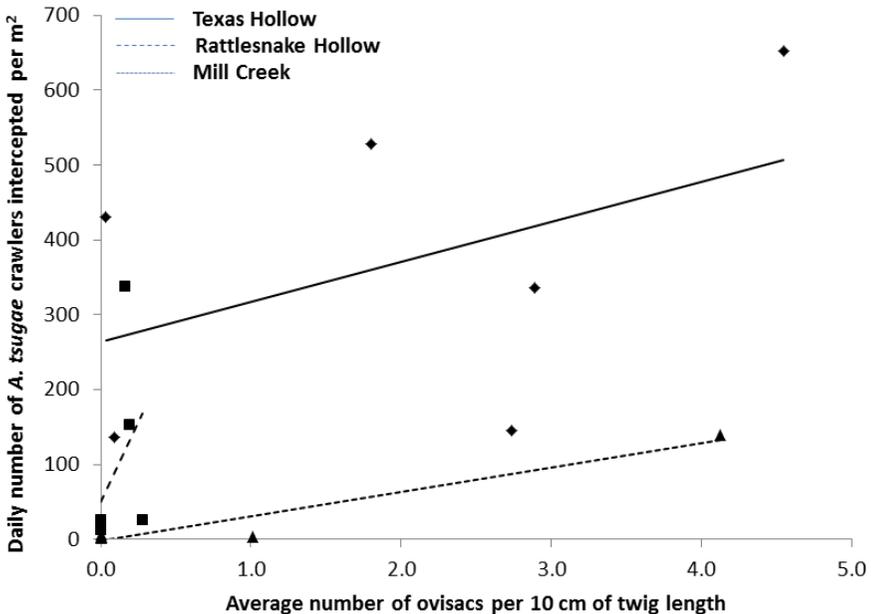


Figure 3. Relationships between daily interception rate of crawlers on sticky traps and abundance of ovisacs sampled in the live crown of eastern hemlock at three sites in upper state New York from May to July 2014. Regressions were not significant for Texas Hollow ($P = 0.37$) and Rattlesnake Hollow ($P = 0.44$) but was significant for Mill Creek (Y = 32.41X − 1.56; $r^2 = 0.92$; $P = 0.001$), where populations of *A. tsugae* were low and traps were placed under hemlock trees that were growing alone or in groups of two or three.

As long as all new infestations of *A. tsugae* uncovered in Canada or jurisdictions in the USA are limited to a few trees, it makes sense to attempt eradication. Within that framework, it is critical to identify all infested trees as soon as possible and treat them to prevent establishment, expansion, and spread of the population. Grids of sticky traps represent an effective and rapidly deployable tool for quality control following treatment. Not only could they be used to determine whether residual populations remain, they could also be used to find out if some have already spread to nearby stands. In addition, sticky traps may prove useful at detection of *A. tsugae* infestations under trees where their live canopy cannot be accessed from the ground by direct means such as visual examination or by pole pruning. However, trapped specimens suspected to be HWA may require identification because other species of adelgid crawlers, such as *Adelges piceae* (Ratzeburg) and *Pineus strobi* (Hartig), could be intercepted on sticky traps when white pine and balsam (or Fraser) fir are present, respectively. These specimens should be cleared and slide mounted for morphological identification or subjected to DNA extraction or both (Annand 1924, Castalanelli et al. 2010), but these methods may not be feasible during survey operations or for small insects, such as *A. tsugae* crawlers, submerged in the adhesive of sticky traps. At a minimum, interception of crawlers that are suspected to be HWA should immediately trigger more intensive surveys within the trapped area. Finally, we suspect that this technique may prove useful at detection and monitoring of the non-native balsam woolly adelgid, *A. piceae*, which is a quarantined pest in western Canada.

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