

## Sampling and Biostatistics

# A funnel trap for the detection of hemlock woolly adelgid (Hemiptera: Adelgidae) and a method of extracting crawlers from trap samples

Jeffrey G. Fidgen<sup>1,\*</sup>, Glen Forbes<sup>1</sup>, Lucas E. Roscoe<sup>1</sup>, Michael Stastny<sup>1,✉</sup>,  
Berni M. van der Meer<sup>1</sup>, Jeffrey Ogden<sup>2</sup>, and Martin Williams<sup>1</sup>

<sup>1</sup>Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre, Fredericton, NB, Canada

<sup>2</sup>Nova Scotia Department of Natural Resources, Shubenacadie East, NS, Canada

\*Corresponding author. Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre, 1350 Regent St. South, Fredericton, NB E3B 5P7, Canada (Email: [jeff.fidgen@nrcan-mcan.gc.ca](mailto:jeff.fidgen@nrcan-mcan.gc.ca)).

Subject Editor: Christopher Fettig

Received on 21 March 2025; revised on 12 May 2025; accepted on 16 June 2025

Eastern hemlock, *Tsuga canadensis* (L.) Carr., in eastern Canada is under threat from the invasive hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae). Early detection is a key feature to the management of *A. tsugae* because the impacts of this pest accrue quickly due to its bivoltine life cycle, and treatments can take a year or more to become effective. We tested a novel funnel trap design to collect the mobile first instar nymphs (crawlers) as a tool for early detection of adelgid infestations prior to host symptoms. The funnel traps performed better at detecting *A. tsugae* crawlers at very low abundance in a stand compared to vertically oriented sticky traps or to canopy branch tip sampling. Satisfactory detection rates for operational surveys were achieved using one or two funnel traps per site deployed for 2 wk during each of the two generations of *A. tsugae* and moving traps to new locations in the stand-between generations. We also optimized a protocol for extracting crawlers from trap samples, using stacked sieves (425 and 100  $\mu$ m) to remove debris and retain crawlers, respectively, with the probability of detecting at least one crawler unaffected by the presence of debris. The improved trapping and extraction technique is aimed at stand-level early detection of this destructive pest and could be adapted to other similar, cryptic insect pests.

**Keywords:** canopy sampling, resampling, environmental DNA

## Introduction

Early detection is a critical component of strategies for protecting crops, forests, and ecosystems from the damaging effects of invasive insect pests. Timely identification of pest threats allows for swift intervention, reducing potential harm by slowing the progress of the infestation (Reaser et al. 2020). Effective monitoring technologies are essential for the early detection of invasive forest pests, particularly when tree symptoms are not easily observed until the pest is well established, after which eradication may not be possible. The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is an example of an invasive pest where its early detection in tree canopies is difficult because of the cryptic nature, and highly patchy initial infestations of this pest over potentially large landscapes (Evans and Gregoire 2007).

The adelgid poses a significant threat to eastern North American hemlock (*Tsuga* sp.) ecosystems. Eastern hemlock, *T. canadensis* (L.) Carr. (Pinaceae), is a late successional tree species and regarded as an ecological foundation species (Ellison et al. 2005, 2015, Parker et al. 2023). Healthy eastern hemlock stands also provide social values including increased property values and recreation (eg Holmes et al. 2010, Aukema et al. 2011, Li et al. 2014, 2022). Hemlock decline and mortality from *A. tsugae* are affecting the integrity and consequently the ecological function of this unique ecosystem in the eastern United States of America (eg Ellison et al. 2010, 2018, Spaulding and Rieske 2010, Witt et al. 2012, Brantley et al. 2013, Stodola et al. 2013, Preisser et al. 2014). In Eastern Canada, hemlock's abundance has already been reduced by three quarters or more since European colonization due to harvesting and land-use

change (Loo and Ives 2003, Snuffling et al. 2003). Under *A. tsugae* invasion in eastern Canada, this unique tree and its ecosystem values are at risk (Emilsson and Stastny 2018).

With its minute size, rapid reproduction via parthenogenesis, and insidious feeding in the canopy, asymptomatic *A. tsugae* populations can often elude detection until they are well established. The adelgid spreads passively over long distances during its mobile stage (first instar nymphs, called crawlers) in both of its two generations (progrediens, sistens), mainly by wind, animals, and human activity (McClure 1990, Russo et al. 2018). The remaining life stages of *A. tsugae* (ie three more instars of nymphs, adults) are sessile on new shoots (and older twigs when new shoots are not available), within a self-made woolly covering called an ovisac (McClure 1987) in which the adults lay their eggs. Although the adelgid has been spreading throughout the United States of America since its discovery in eastern Virginia in the early 1950s, this pest is a relative newcomer to eastern Canada. First discovered in Ontario (Etobicoke) in 2012, *A. tsugae* has since been detected at several locations within that province (CFIA 2024). The adelgid was found in southwestern Nova Scotia (2017) (for a review see MacQuarrie et al. 2025) but based on the impact and extent of its infestations, it likely established up to a decade earlier. With warmer winters due to climate change, *A. tsugae* will continue to spread throughout the range of eastern hemlock in Canada (Emilsson and Stastny 2018, Jeong et al. 2024). An untreated infested hemlock has a high probability of dying, though the timing of mortality varies considerably (3 to 15 yr; McClure 1991, Ellison et al. 2010).

Incipient populations of *A. tsugae* often establish in the upper canopy, away from the notice of visual surveys (Evans and Gregoire 2007). Therefore, early detection techniques must target the upper canopy, which in mature stands presents a significant challenge for traditional forestry survey techniques such as branch sampling with pole pruners (McClure 1990, Fidgen et al. 2019, Sanders et al. 2023). Two other ground-based approaches can be used to detect incipient populations of *A. tsugae* in the upper canopy of tall trees. The first is ball sampling, which is an active method where Velcro-covered balls are shot into the canopy with a slingshot to capture adelgid wool upon contact with ovisacs (Fidgen et al. 2019). The second is trapping, which relies on a passive collection of crawlers and other adelgid life stages near ground level when they are dislodged from the canopy (McClure 1990, Fidgen et al. 2020, Sanders et al. 2023). Detection efficiency with traps is related to the surface area of traps at a location (ie trap size and/or number of traps per site) (Fidgen et al. 2020, Saunders et al. 2023), in addition to their placement in locations with a high probability of *A. tsugae* establishment, such as stand edges or under the crowns of trees above average canopy height (Costa and Onken 2006). Traps without a sticky substance are preferred for ease of handling the samples, or when using molecular methods to detect *A. tsugae* environmental DNA (eDNA) (Kirtane et al. 2022, Sanders et al. 2023).

Recently, a new passive trapping technique for the detection of *A. tsugae* was reported by Sanders et al. (2023) who used an 8-section Lindgren funnel trap. While effective, this trap—originally designed to intercept adult scolytid beetles (Lindgren 1983)—has some drawbacks: its multiple funnels add weight, a closed top reduces the vertical entry of crawlers, and rebar may be needed for deployment. Therefore, we set out to design a single-funnel trap protocol, aiming to improve its operational ease of use. We then tested its effectiveness in detecting *A. tsugae* crawlers at sites with a range of adelgid abundance. In this study we also assessed (i) the duration of trap deployment required to detect low-level infestations and (ii) the minimum number of traps per site to accomplish detection, as determined

using a bootstrapping approach. We also compared the detection rates using the new funnel traps with those obtained through branch tip sampling and deployment of sticky traps, two methods presently in use to detect and monitor *A. tsugae*.

Because the usefulness of traps often hinges on the efficient and accurate sorting of their contents, we also developed and optimized a method of extracting crawlers from the trap samples. The protocol we used to detect *A. tsugae* crawlers was inspired by techniques used to extract overwintering second-instar larvae of the spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae) from chopped branch samples (Miller et al. 1971, Hartling 1994). We present this modified filtering and extraction technique for *A. tsugae*, conducting a series of tests to maximize the recovery of crawlers and to evaluate the impact of debris in a sample on the accuracy of detection as [Supplementary Material](#). As a last step, we assessed the likelihood of obtaining a false positive field sample due to contamination of the filtration equipment with crawlers from a previous sample. In combination with the redesign of the funnel trap, this methodology aims to improve early detection of *A. tsugae* at low infestation levels when other techniques may be ineffective and could be adapted for trapping of similar insects when they are dislodged from the tree canopy.

## Materials and Methods

### Trapping System

A trap resembles a large funnel, consisting of three components ([Fig. 1A](#)). The main component is a 20-cm wide funnel section equipped with a wet collection cup. To increase the collection surface area, we added a 42.7-cm wide (1,431 cm<sup>2</sup> intake surface) ‘Allison’ collar (Allison et al. 2014) above the funnel section (canopy pan trap, Synergy Semiochemicals Corp., Delta, British Columbia). The Allison collar was covered by a square sheet of hardware cloth (55 × 55 cm, 5 × 5 mm mesh size) to keep large debris out of the trap. Prior to hanging a trap, the cups were filled with 200 ml of an equal part solution of propylene glycol and water, with 0.1 ml of detergent added to break surface tension and allow all catch to pass to the bottom of the cup. Each trap was hung approximately 4 m off the ground on a lower canopy hemlock branch by an R-shaped wire hook attached to the trap (Midwest Wire Products LLC, Sturgeon Bay, WI). This hook was connected by a two-strand braided wire to a Y-shaped spreader that connected to straps passing through the Allison collar and funnel section ([Fig. 1A](#)). Traps were installed using sectional poles equipped with a threaded wire hook, originally used to hang prism traps for the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae).

### Field Testing

In 2022, we selected eight sites in southwestern Nova Scotia, selected by their low *A. tsugae* infestation levels, to test the new trap design ([Fig. 2](#)). The stands were very large, but we focused on the portion of the stands dominated by young (80 to 120 yr) eastern hemlock (50% or more), mixed mostly with white pine (*Pinus strobus* L.) and red spruce (*Picea rubens* Sargent) with scattered red maple (*Acer rubrum* L.) and trembling aspen (*Populus tremuloides* Michx.). We sampled the hemlock canopy to estimate infestation levels of *A. tsugae* within the portion of the stand surrounding the traps, using pole pruners to cut one mid-crown branch (30 cm length) per tree. On sampled branches, we determined the proportion of infested shoots by counting the number of new shoots infested with at least one adelgid ovisac out of the total number of new shoots present on the



**Fig. 1.** A. Modified funnel trap, called the Synergy Semiochemicals canopy pan trap, used to collect *Adelges tsugae* crawlers (first instar nymphs) in infested *Tsuga canadensis* stands in southwestern Nova Scotia, Canada. B: Extracted trap sample on gridded filter paper, after a Buchner funnel under vacuum created 219 piles of debris and *A. tsugae* crawlers. Red cells are an example of the 10-cell, X-shaped subsample of the filter paper. C: Close-up view of one pile consisting of debris and crawlers.

branch tip. We sampled 25 haphazardly selected hemlocks in May and repeated the process again in July, for a total of 50 trees sampled per site (Table 1). Except for Site 4, which was a heavily infested site and served as a positive control in the experiment, all sites had a range of low abundance of adelgids and no evidence of hemlock decline, such as needle loss, reduced shoot production, and crown thinning associated with higher infestation. The ovisacs assessed during the May and July canopy samples would eventually produce progrediens and sistens crawlers, respectively. Therefore, infestation levels estimated from canopy sampling (proportion of shoots with at least one ovisac) in May and in July were compared to the presence of crawlers in trap samples collected in May and June and in July and August, respectively.

We set three traps at each site, equidistant along a 100-m transect that paralleled a stand edge (roadside edge or edge formed by a hemlock and non-hemlock stand), with deployment beginning 9 May 2022 and ending 3 August 2022. Trap samples were collected several times during this period, allowing us to compare the effect of varying length of time of trap deployment, called trapping periods (Supplementary Table S1). For all trapping periods, the contents of each trap were collected and stored separately. For the first collection (May 9 to 25), the trap samples were collected by pouring the contents through a 190  $\mu$ m paper paint strainer (RAMPRO, Newburg, NY). We then placed the strainer with contents inside a 50-ml Falcon High Clarity Conical Centrifuge Tube (Thermo Fischer Scientific, Waltham, MA) and topped the tube up with fresh propylene glycol solution. For the remaining collections, each trap sample was poured in its own 500 ml plastic jar.

Because some of our field samples were likely to contain several thousand crawlers due to the high abundance of *A. tsugae* in the canopy (ie Sites 2 and 4; Table 1), we performed additional ‘blank’ washes in between processing field samples. Here we thoroughly rinsed the sieves and Buchner funnel in between field samples. To check whether crawlers remained in the sieves or Buchner funnel after cleaning the equipment, we followed the same steps for

processing a trap sample as described above and examined the filter paper for crawlers. We did not process a field sample until the equipment was confirmed free of crawlers from the previous sample, performing two blank washes after processing each field sample.

### Optimizing Trapping Method

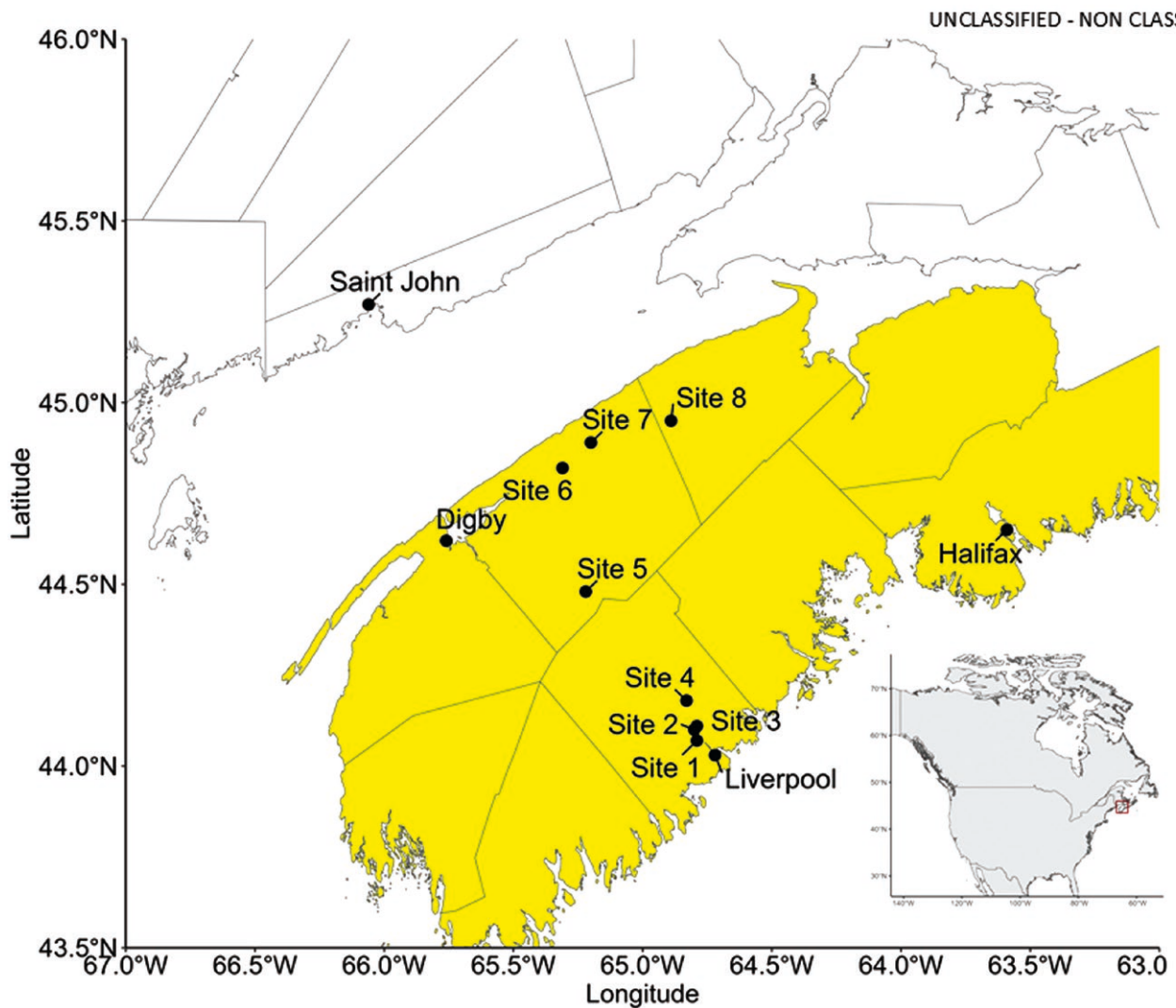
We examined three factors to help optimize the trapping method: (i) the number of traps deployed per site; (ii) the length of the trapping period; and (iii) the sensitivity of the traps when used in stands with low abundance of *A. tsugae*.

To evaluate whether the detection accuracy varied with the length of the trapping period, we assessed the proportion of traps positive after 0.5-, 1.5-, 2-, and 3-wk periods in the field from May to early August at all sites (Supplementary Table S1). For each site and trapping period, we pooled the number of positive traps out of the total number of traps present during the first three collection periods for the progrediens crawler stage and the next three collection periods for the sistens crawler stage. We then calculated the proportion of positive traps in each generation by dividing the number of positive traps by the total number of traps per site. We compared these proportions to the corresponding estimate of the proportion of shoots infested at each site, as determined by canopy branch tip sampling.

### Sticky Trapping

We compared the detection of crawlers with funnel traps versus sticky cards. For this, we deployed three double-sided, yellow sticky traps per site, one trap in a vertical orientation near each of the funnel traps. Traps were installed for three trapping periods (Supplementary Table S1), the first during the progrediens crawler stage and the next two during the sistens crawler stage. The traps were 15 cm wide  $\times$  18 cm long, yellow, wax-coated cardboard sheets with a sticky coating applied to both sides, giving the trap a 540 cm<sup>2</sup> collection surface area (Alphascent Double-sided Yellow Sticky Card, Alpha Scents Inc., Canby, OR). The traps were assessed as





**Fig. 2.** Sites in southwestern Nova Scotia with funnel traps deployed to detect *Adelges tsugae* crawlers (first instar nymphs) falling from the canopy of *Tsuga canadensis*. Shaded counties currently contain infestations of *A. tsugae*.

**Table 1.** Estimated levels of *Adelges tsugae* infestation (proportion of *Tsuga canadensis* shoots infested with at least one ovisac,  $P(\text{shoots})$ ), at eight sites in southwestern Nova Scotia in 2022, obtained through canopy sampling 25 trees at each of two time points corresponding to the two adelgid generations. Estimate of  $P(\text{shoots})$  at Site 4 was based on a limited sample of branch tips due to poor new shoot production because of heavy *A. tsugae* infestation.

Site	$P(\text{shoots})$ May	$P(\text{shoots})$ July
1	0.0	0.002
2	0.0004	0.21
3	0.0	0.0
4	0.33	0.50
5	0.03	0.007
6	---	0.004
7	0.02	0.03
8	0.0	0.0

described in Fidgen et al. (2020), using a dissection microscope to scan both sides of the cards for crawlers. We recorded whether the sticky trap contained at least one adelgid crawler or not.

### Crawler Extraction and Quantification

In the laboratory, samples from the funnel traps were poured through stackable sieves (100  $\mu\text{m}$  below a 425  $\mu\text{m}$  sieve) to separate crawlers from debris and the propylene glycol solution (for context see Supp., Development of crawler extraction technique). After spraying the top sieve to dislodge crawlers, all material caught on the lower, 100  $\mu\text{m}$  sieve was rinsed into a jar. Contents of the jar were then poured over gridded filter paper in a 15-cm diameter Buchner funnel under vacuum pressure to remove water from crawlers and similar-sized debris. Using a dissecting microscope, we counted all crawlers found on the filter papers (one filter paper per trap sample) to quantify the occurrence of crawlers over the season at each site. The Buchner funnel produced 219 piles of crawlers and similar-sized debris on the filter paper (Fig. 1B). We counted the number of crawlers in each pile in turn until all piles had been examined. Crawler counts were then divided by the number of days of the trapping period which varied over the season (ie 3 to 21 d, or 0.5 to 3 wk) (Supplementary Table S1). To test whether a subsample of the filtered sample would provide similar accuracy, we counted the crawlers in the piles in 10 full-sized grid cells (~50 piles) in the center of the filter paper in an 'X' pattern (Fig. 1B). For this comparison, we recorded whether the full sample or subsample of each filter paper was positive for crawlers or not (Fig. 1C).

## Statistical Analysis

### Field Testing

We fit a binomial general linear model (GLM) to test for the effect of subsampling the piles as compared to sampling all piles on the filter paper using the proportion of adelgid positive filter papers as the response variable. We fit a binomial GLM to test for the effect of trapping period length on the proportion of traps positive for adelgids as the response variable. We used logistic regression to evaluate the effect of trap type and adelgid generation on the proportion of positive traps per site, as the response variable, using the proportion of shoots infested with at least one ovisac in the stand as a covariate.

Data were analyzed in the R statistical computing environment, version 4.3.0 (R Core Team 2023). GLMs and the logistic model were fit using functions in the 'stats' package (R Core Team 2023). When treatments were significant (ie  $P \leq 0.05$ ) differences amongst the levels of the treatments were evaluated using the 'emmeans' package (Lenth 2023). We tested the assumptions of our models using residuals plots and dispersion parameters according to Zuur et al. (2009).

### Bootstrapping

We simulated trapping a hypothetical population of *A. tsugae* to evaluate detection rates when one or two traps were deployed per stand instead of three. We assumed complete stand coverage (ie successful detection) was achieved with three traps; however, only one of the three trap locations was positive for the adelgid. If a trap was placed in that location, it always detected the adelgid infestation. The bootstrapping approach was based on sampling without replacement (Legg et al. 2014), with 500 sampling iterations for each scenario and generation of *A. tsugae* sampled. The first scenario involved the deployment of a single trap in the hypothetical stand for each generation of *A. tsugae*. All bootstrapping was performed in Microsoft Excel using a function to randomly pick one of the three locations (a matrix consisting of 1, 0, 0) in the stand, and then the function returned the value for that location to a new column, with a 1 equal to detection of the infestation. We counted the number of iterations where 1 was returned out of the 500 iterations and computed a percentage of iterations where the hypothetical population of the first-generation (progrediens) crawlers was detected. Then, for detection of the second-generation (sistens) crawlers, we repeated the simulation; however, we only performed bootstrapping of the iterations that were 0 after the first round of bootstrapping (ie where progrediens crawlers were not detected). To do this, we reduced the matrix by eliminating one of the 0 values from the matrix (1, 0) to simulate moving the trap to one of the other two locations. We added the values for each stage and counted the number of iterations that returned a value of 1: this count was divided by 500 and from that value, we computed the percentage of iterations where the infestation was detected. In the second scenario, we simulated the deployment of two traps in the stand, using the same approach as described above. However, we did not run the function for sampling the sistens generation of crawlers because, with the reduced matrix, the probability of detecting sistens crawlers with two traps was 1 as the next deployment would always include the only remaining unsampled—and positive—location.

## Results

### Field Testing

We counted from 0 to 3,539 crawlers per funnel trap per day (total count = 113,589 crawlers). Traps collected the most crawlers at Site

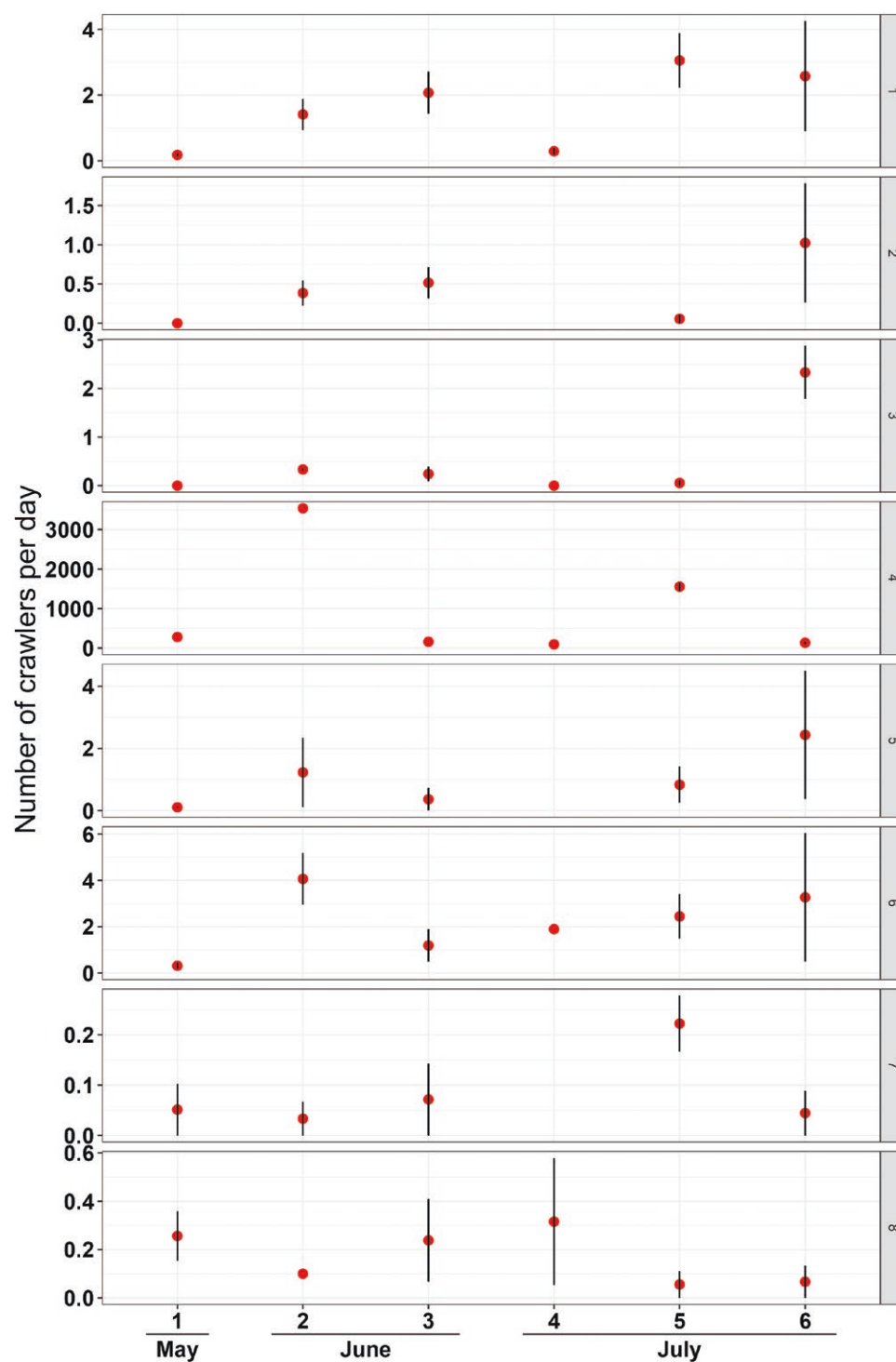
4, with two distinct peaks in abundance at that site occurring in early June and again in mid-July (Fig. 3). There were similar peaks in crawler abundance at the other sites with peaks 4–8 wk apart. Although we detected crawlers at all sites, not all trapping periods detected the adelgid. We did not detect crawlers at Site 2 in the May 9–25 trapping period; and at Site 3 at the May 9–25 and June 21–Jul 12 trapping periods (Fig. 3). When subsampling the filter paper, we detected crawlers significantly less often (51%) compared to sampling all of it (78%) ( $D = 29.42$ ;  $df = 1$ , 208;  $P < 0.001$ ). The blank washes were useful to reduce the false positive rate when processing subsequent field samples. Of the 113,589 crawlers we recovered, only 11 crawlers were recovered from the first (9 [8 Site 4, 1 Site 6]) and second (2 [Site 4]) blank washes.

The array of three traps detected the adelgid at all sites in both the progrediens and sistens generations. However, in eight out of 40 instances amongst all trapping periods only one trap out of the three at a site detected the adelgid. Had we had set fewer traps per site, there would be an increased risk of missing the infestation (false negative). Expanding on this, the bootstrapping found that the percentage of positive iterations, when only one trap is set per stand during the peak progrediens crawler stage in June, was 34%. When one trap was set in a stand in one of the two remaining locations for sistens crawlers, the percentage of positive iterations increased to 69%. We observed a similar trend when using two traps per site instead of one. For two traps, 65% of the iterations were positive for detecting progrediens crawlers, and all the negative iterations became positive when sampling sistens crawlers.

The trapping period length influenced the detection of crawlers ( $D = 14.18$ ;  $df = 4$ , 153;  $P = 0.007$ ), but we only detected significant differences between the 0.5 vs 2-wk and 0.5 vs. 3-wk treatments, with the longer duration resulting in doubling of the proportion of positive detections (Fig. 4). The proportion of positive traps was significantly higher for funnel traps than for sticky traps ( $D = 0.45$ ;  $df = 1$ , 28;  $P = 0.004$ ), particularly when the abundance of the adelgid in the stand was low (Fig. 5). For both trap types, the proportion of positive traps increased with increasing proportion of infested shoots at a site ( $D = 0.58$ ;  $df = 1$ , 26;  $P = 0.001$ ). The funnel traps detected the adelgid at all sites although at least two trapping periods were needed at Sites 2 and 3 to detect the infestation (Fig. 3). In comparison, sticky traps and branch sampling were unable to detect the adelgid at Sites 3 and 8 despite at least two attempts at detection for each method (Fig. 5, Table 1).

## Discussion

Early detection of *A. tsugae* in the hemlock canopy is key to its efficient management because populations often begin there and are highly patchy, yet populations of this pest build quickly and can cause damage to trees in as early as 3 yr in Nova Scotia (J. Ogden, pers. obs.). In this study, we focused on the improvement of trap technology by testing a new trap design with a large intake surface area used to collect *A. tsugae* crawlers as they dislodge from the hemlock canopy. Earlier studies showed that the surface area of traps in a stand influences the probability of detecting *A. tsugae* (Fidgen et al. 2020, Sanders et al. 2023). For example, with two traps per site and intake surface area of 1,431 cm<sup>2</sup> per trap, this would be equivalent to seven sticky cards set up in a horizontal orientation (Fidgen et al. 2020). Our novel trapping system (i) collects *A. tsugae* crawlers when crawlers are present in the stand at very low density; (ii) is relatively lightweight and thus easily deployed; and (iii) employs an efficient protocol for processing trap samples. Only a handful of early detection techniques work well for *A. tsugae* in the

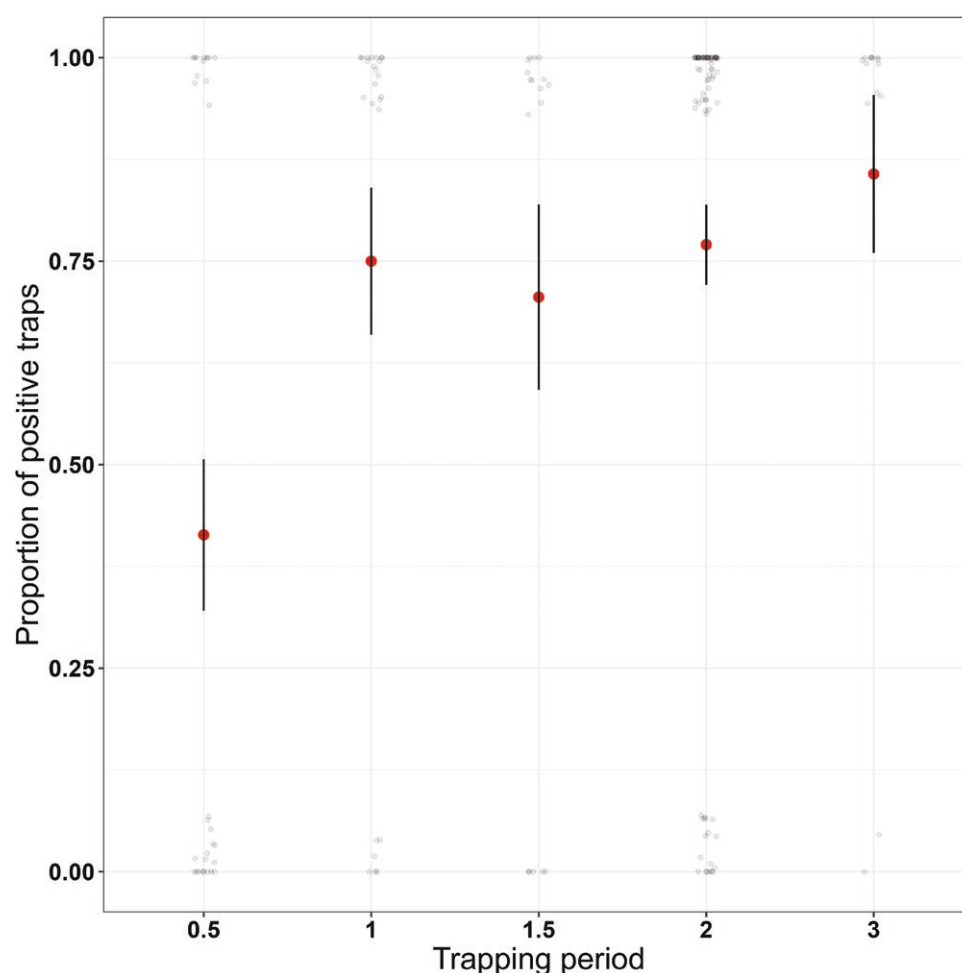


**Fig. 3.** Number (mean  $\pm$  SE) of *Adelges tsugae* crawlers (first instar nymphs) caught per day in eight *Tsuga canadensis* stands (Sites 1-8) in southwestern Nova Scotia over six trapping periods spanning 3 mo. See text for further details.

high canopy of mature hemlock stands (ie traps, ball sampling, and possibly remote sensing [eg Williams et al. 2017]). These stands are arguably some of the highest-value hemlock forests to conserve from the perspective of the ecosystem services (eg Rohr et al. 2009, Ellison et al. 2015, Chisholm and Gray 2024), wildlife habitat (eg Yamasaki et al. 2000), and human appreciation they provide.

A new detection tool ought to improve early detection of a pest population or have a detection rate comparable to existing

techniques but with better efficiency. Information on whether the funnel traps can detect incipient infestations of *A. tsugae* sooner or are more efficient than existing techniques in the Nova Scotia context is presently lacking. We are presently evaluating the efficiency of funnel traps against other methods of detecting *A. tsugae* infestations (unpublished data). In hemlock stands dominated by tall, mature trees with little understory hemlock, early detection of low-density infestations in the upper canopy is likely to be considerably more

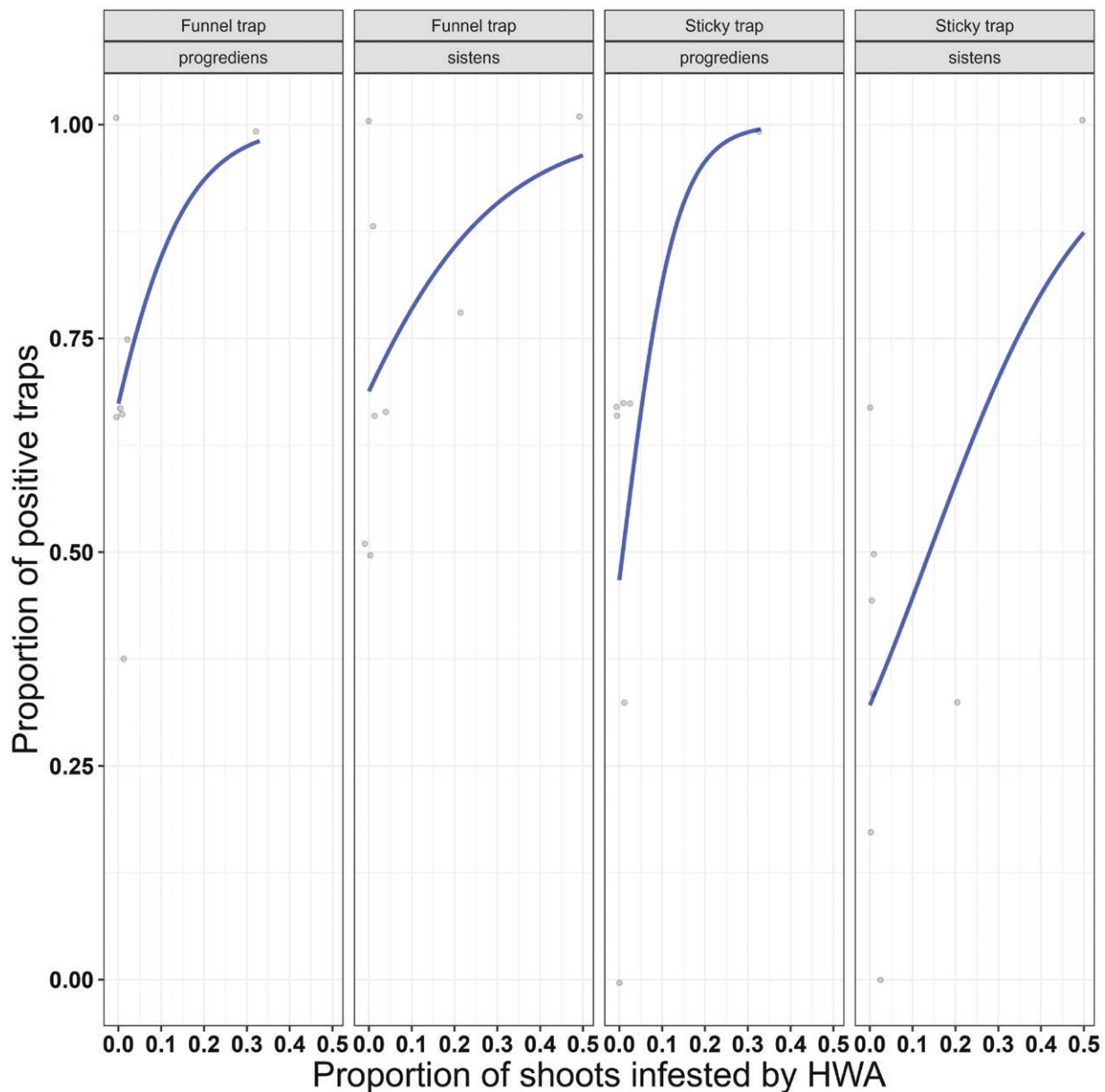


**Fig. 4.** Mean proportion ( $\pm$  SE) of traps positive for *Adelges tsugae* crawlers (first instar nymphs) when funnel traps were deployed from a half to 3 wk under *Tsuga canadensis* canopies. The proportion of positive traps was significant for the comparisons of 0.5 vs. 2-wk and 0.5 vs. 3-wk, all other comparisons were not significantly different. Circles are raw data that have been offset for visualization. See text for further details.

difficult to achieve through branch sampling than by using funnel traps. Consideration of stand characteristics, some of which can also help in prioritization of high-value hemlock habitat (eg old-growth stands), should be included in deciding which detection survey technique may be most appropriate across a range of stand types, given the inherent limitations and effort involved with each approach.

The funnel trap and extraction methods could likely be adapted for other insect pests that present similar challenges to detection and monitoring, such as red pine scale (*Matsucoccus matsumurae* Bean and Godwin) (Homoptera: Margarodidae) (McClure 1977) or the balsam woolly adelgid (*Adelges piceae* [Ratzburg]) (Hemiptera: Adelgidae) (Arthur and Hain 1984). Both insects have a mobile crawler stage that can dislodge from the canopy to ground level. For example, the invasion of red pine scale is of particular concern for red pine (*Pinus resinosa* Ait.) in eastern Canada, and early detection will be key for effective management response. Our techniques for the processing of trap samples offer flexibility for adaptation to insects of other body sizes by changing the mesh size of the sieves. Indeed, we did occasionally trap crawlers of other adelgid and scale species in the funnel traps that could be visually distinguished from *A. tsugae* crawlers. Ongoing studies with these traps are showing high correspondence between the count of crawlers presumed to be *A. tsugae* and molecular signal of *A. tsugae* DNA in the sample (unpublished data).

As demonstrated in this paper, the funnel traps also offer a tool for yearly monitoring of adelgid populations, and for post-treatment assessments. For example, we observed two peaks in crawler abundance that occurred in June and July at most sites, which corresponds to the phenology of the two *A. tsugae* crawler periods in southwestern Nova Scotia (Roscoe et al. unpublished data). The detection of crawlers with the funnel traps is more sensitive at low abundance of *A. tsugae* in the stand as compared to the sticky traps (Fig. 5). The vertical orientation of the sticky traps is comparable in principle to that of the Lindgren funnel trap used in Sanders et al. (2023), in the sense that for this design to be effective, crawlers would need to be moving somewhat horizontally to be trapped in or on a vertically orientated collection surface. The greatly reduced capture of crawlers on sticky traps compared to funnel traps, manifested by a reduced proportion of positive traps in stands with low infestation levels (Fig. 5), suggests that a horizontal orientation of a trap is superior to a vertical design for collecting crawlers. Albeit passive, traps likely collect crawlers from a large swath of canopy overhead, suggesting they are more efficient as detection tools than sampling branch tips from the canopy (Table 1). In many cases, we were unable to reach the middle or high canopy of hemlock with pole pruners, excluding a significant volume of foliage—with the greatest number of new shoots—from sampling. However, despite the inconsistent results of canopy branch tip sampling, it remains an



**Fig. 5.** Proportion of funnel and sticky traps positive for *Adelges tsugae* crawlers (first instar nymphs) for multiple collection periods during each adelgid generation, as a function of increasing infestation level of *Tsuga canadensis* (proportion of new shoots with at least one *A. tsugae* ovisac), as estimated through canopy sampling with pole pruners. A sticky trap was set in a vertical orientation near each funnel trap.

important, direct tool to confirm *A. tsugae* in a stand, as well as for more detailed observations and tree-level sampling.

Unlike with canopy or ball sampling, the exact source of crawlers in a trap is unknown and may explain the poor correspondence between the detection with trapping versus canopy sampling. The traps may have been placed in an uninfested portion of the stand yet collect crawlers when they are dislodged by wind or animals from other parts of the stand, or from adjacent, unsampled stands. The collection radius of the traps is unclear, although it may be hypothesized from earlier work by McClure (1990), who assessed the spread of adelgid crawlers from an infested stand in Connecticut. In that study, sticky cards were placed in a horizontal orientation at increasing distances downwind from the hemlock stand in a deciduous forest: 93% of the crawlers were recovered on the traps within 300 m of the hemlock stand. We suspect the spread of crawlers would be more

impeded in conifer stands that are more sheltered from winds; in those settings, 100 to 150 m may be a more likely spread estimate for airborne crawlers (J. Fidgen pers. obs.). With this assumption, the potential sampling area of each trap may be approx. 3–7 ha. However, this estimate ignores the small but non-zero probability of interception of crawlers from long-distance dispersal, known to drive the spread of propagules of invasive species (Kot et al. 1996), especially in organisms with high reproductive rates such as *A. tsugae*.

Based on our bootstrapping approach, one or two traps per site appear sufficient for detection of *A. tsugae* crawlers. Of note here is that surveyors must strike a balance between trapping intensity in one generation of the adelgid versus the cost of sampling twice or more a season. Though merely a simulation, two aspects of the bootstrap process need to be highlighted. First, the detection rate increased during the trapping of the second adelgid generation. In



years of high weather-related mortality of the overwintering sistens (MacQuarrie et al. 2024), which is common in southwestern Nova Scotia, the enhanced survival of progrediens on available foliage begins to rebound the population; therefore, trapping for sistens crawlers increases the probability of detection. Second, the detection rate for sistens crawlers might be improved by moving the trap(s) to new locations deemed high-risk for adelgid establishment, such as roadside/riparian trees and windward edges (Costa and Onken 2006), following a negative result when trapping for progrediens crawlers. Moving the trap(s) increases the total sampled area over the season; we recommend new locations at least 100 to 150 m distant to locations used for progrediens trapping.

The duration of trap deployment also influenced detection rates. Although the only significant difference occurred between the shortest (0.5-wk) versus 2-wk and 3-wk periods, the decision about deployment duration should be guided by the purpose of the traps. For example, when detection of incipient infestations is the main objective, longer trapping periods will maximize the probability of detection, albeit at the expense of a higher volume of debris in the trap. On the other hand, when monitoring of higher infestations is the focus, a 1-wk period is likely sufficient, while reducing the debris. With this guidance, it becomes possible to tailor the trapping approach to suit the desired survey objectives.

Sanders et al. (2023) were the first to assess a funnel trap design for the detection of *A. tsugae* crawlers. The Lindgren trap was highly effective compared to other traps they evaluated, but their study did not test this design for the detection of adelgids when present at very low density. Our trap design is likely more effective because it may be more effective at intercepting crawlers falling from the canopy at low wind speeds. For example, the sticky traps (our surrogate trap for the Lindgren trap) had  $2.6 \times$  less surface area than the funnel traps but collected  $78 \times$  fewer crawlers per day than the funnel traps (unpublished data). Several other, practical features of the funnel traps offer improvement over the Lindgren design. Their light weight (~1 kg) allows installation higher above ground, reducing the chances of damage by large animals, without requiring rebar or ropes. Lastly, the hardware cloth screen that reduces debris accumulation can be omitted; it adds about 30% more weight and an extra component to be cleaned (unpublished data).

Because the detection of crawlers on the filter paper with debris is tedious, we examined the option of using a subsample (20% portion of the filter cells); this proved significantly less likely of detecting crawlers, that is, when they are present at low density on the filter papers. A suitable approach is to examine all piles until the first crawler is found or all piles have been examined. We also investigated the possibility of false positives in our sample processing. Trap samples may contain several thousand crawlers, increasing the likelihood that subsequent samples are contaminated with crawlers that remain on the processing equipment after cleaning. Ensuring that the filtration systems are clear before the next sample is crucial to avoid the potential deployment of resources to conduct follow-up assessments at false-positive sites. Our solution to this involved 'blank' extractions; usually, only one blank extraction was needed to confirm the sieves were clean for the next sample. Operationally, blank extractions need assessment as soon as possible to clear the equipment for the next field sample. That said, if the primary use of this trap is for early detection of incipient, low-level infestations, false positives are much less likely after a first blank wash. We only had one instance where a second blank wash was positive, at a site where the high infestation could be detected visually without any tools (ie Site 4).

We suspect the funnel traps are suitable for collecting *A. tsugae* environmental DNA (eDNA) from the hemlock canopy. We are

presently developing and refining protocols to recover DNA from these traps and are testing for contamination at every step in the process to develop a system where traps stay clean from field deployment to lab processing and analysis. Because there are other inputs of *A. tsugae* DNA falling from the canopy, such as older nymphs, wool, and eggs, the use of these traps could be applicable outside of the active crawler periods of *A. tsugae*. Likewise, these traps make possible the detection and monitoring of the eDNA of predators being released for biological control of *A. tsugae* (Kirtane et al. 2022, Mayfield III et al. 2023, Sanders et al. 2023, Liu et al. 2024). Assessment of trap samples using molecular tools will improve the efficiency of processing trap samples as compared to visually examining filter papers, and with appropriate adjustments to the extraction method could broaden the use of this funnel trap for molecular detection of other insects.

## Supplementary material

Supplementary material is available at *Journal of Economic Entomology* online.

## Acknowledgments

We thank M. Doyle, A. McGill, and the field staff of the Nova Scotia Department of Natural Resources (NSDNR) for assistance with data collection. We thank Mersey River Chalets and the NSDNR for permission to use study sites. We also thank T. Swanburg for reviewing earlier versions of the manuscript and Bob Setter for helpful discussion on trap development. Funding for this work was provided through SERG-I and by Natural Resources Canada, Canadian Forest Service.

## Author contributions

Jeffrey Fidgen (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Methodology [equal], Writing – original draft [lead]), Glen Forbes (Conceptualization [equal], Data curation [equal], Methodology [equal], Writing – review & editing [equal]), Lucas E. Roscoe (Funding acquisition [lead], Project administration [equal], Resources [equal], Supervision [equal], Writing – review & editing [equal]), Michael Stastny (Formal analysis [equal], Visualization [equal], Writing – original draft [equal], Writing – review & editing [equal]), Berni van der Meer (Data curation [equal], Investigation [equal], Methodology [equal], Writing – review & editing [equal]), Jeffrey Ogden (Methodology [equal], Resources [equal], Writing – review & editing [equal]), and Martin Williams (Conceptualization [lead], Data curation [equal], Funding acquisition [equal], Investigation [equal], Methodology [equal], Writing – original draft [equal], Writing – review & editing [equal])

*Conflicts of interest.* None declared.

## References

- Allison JD, Bhandary BD, McKenney JL, et al. 2014. Design factors that influence the performance of flight and intercept traps for the capture of longhorned beetles (Coleoptera: Cerambycidae) from the subfamilies Lamiinae and Cerambycinae. *PLoS One* 9:e93203. <https://doi.org/10.1371/journal.pone.0093203>
- Arthur FH, Hain FP. 1984. Seasonal history of the balsam woolly adelgid (Homoptera: Adelgidae) in natural stands and plantations of Fraser fir. *J. Econ. Entomol.* 77:1154–1158. <https://doi.org/10.1093/jeet/77.5.1154>
- Aukema JE, Leung B, Kovacs K, et al. 2011. Economic impacts of non-native forest insects in the continental United States. *PLoS One* 6:e24587. <https://doi.org/10.1371/journal.pone.0024587>

- Brantley S, Ford CR, Vose JM. 2013. Future species composition will affect forest water use after loss of eastern hemlock from southern Appalachian forests. *Ecol. Appl.* 23:777–790. <https://doi.org/10.1890/12-0616.1>
- Canadian Food Inspection Agency (CFIA). Questions and answers: hemlock woolly adelgid (*Adelges tsugae*) detection. 2024. [accessed December 2024]. <https://inspection.canada.ca/en/plant-health/invasive-species/insects/hemlock-woolly-adelgid/questions-and-answers>
- Chisholm PJ, Gray AN. 2024. Forest carbon sequestration on the west coast, USA: Role of species, productivity, and stockability. *PLoS One* 19:e0302823. <https://doi.org/10.1371/journal.pone.0302823>
- Costa S, Onken B. Standardizing sampling for detection and monitoring of the hemlock woolly adelgid in eastern hemlock forests. USDA Forest Service, Forest Health Technology Enterprise Team. FHTET-2006-16, 2006.
- Ellison AM, Bank MS, Clinton BD, et al. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Front. Ecol. Environ.* 3:479–486. [https://doi.org/10.1890/1540-9295\(2005\)003\[0479:lofscf\]2.0.co;2](https://doi.org/10.1890/1540-9295(2005)003[0479:lofscf]2.0.co;2)
- Ellison AM, Barker-Plotkin AA, Foster DR, et al. 2010. Experimentally testing the role of foundation species in forests: the Harvard Forest hemlock removal experiment. *Methods Ecol. Evol.* 1:168–179. <https://doi.org/10.1111/j.2041-210X.2010.00025.x>
- Ellison AM, Barker-Plotkin AA, Khalid S. 2015. Foundation species loss and biodiversity of the herbaceous layer in New England Forests. *Forests* 7:1–12. [10.3390/f7010009](https://doi.org/10.3390/f7010009)
- Ellison AM, Orwig DA, Fitzpatrick MC, et al. 2018. The past, present, and future of the hemlock woolly adelgid (*Adelges tsugae*) and its ecological interactions with eastern hemlock (*Tsuga canadensis*) forests. *Insects* 9:172. <https://doi.org/10.3390/insects9040172>
- Emilson CE, Stastny M. 2018. A decision framework for hemlock woolly adelgid management: review of the most suitable strategies and tactics for eastern Canada. *Forest Ecol. Manag.* 444:327–343. <https://doi.org/10.1016/j.foreco.2019.04.056>
- Evans AM, Gregoire TG. 2007. The tree crown distribution of hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae) from randomized branch sampling. *J. Appl. Entomol.* 131:26–33. <https://doi.org/10.1111/j.1439-0418.2006.01121.x>
- Fidgen JG, Fournier RE, Whitmore MC, et al. 2019. Factors affecting Velcro-covered balls when used as a sampling device for wool of *Adelges tsugae* (Hemiptera: Adelgidae). *Can. Entomol.* 151:101–114. <https://doi.org/10.4039/tce.2018.50>
- Fidgen JG, Whitmore MC, Studens KD, et al. 2020. Sticky traps as an early detection tool for crawlers of *Adelges tsugae* (Hemiptera: Adelgidae). *J. Econ. Entomol.* 113:496–503. <https://doi.org/10.1093/jee/toz257>
- Hartling LK. Reference document on forecasting spruce budworm infestations. New Brunswick Department of Natural Resources. Fredericton, NB, Canada, 1994.
- Holmes TP, Murphy EA, Bell KP, et al. 2010. Value impacts of hemlock woolly adelgid in residential forests. *Forest Sci.* 56:529–540. <https://doi.org/10.1093/forestscience/56.6.529>
- Jeong YS, Lee D-S, Lee D-Y, et al. 2024. Predicting potential occurrence of *Adelges tsugae* (Homoptera: Adelgidae) on a global scale under climate change scenarios using maximum entropy model. *Global Ecol. Conserv.* 50:e02861. <https://doi.org/10.1016/j.gecco.2024.e02861>
- Kirtane A, Dietschler NJ, Bittner TD, et al. 2022. Sensitive environmental DNA (eDNA) methods to detect hemlock woolly adelgid and its biological control predators *Leucotaraxia* silver flies and a *Laricobius* beetle. *Environmental DNA* 4:1136–1149. <https://doi.org/10.1002/edn3.317>
- Kot M, Lewis MA, van den Driessche P. 1996. Dispersal data and the spread of invading organisms. *Ecology* 77:2027–2042. <https://doi.org/10.2307/2265698>
- Legg DE, Fidgen JG, Ryall KL. 2014. Resampling simulator for the probability of detecting invasive species in large populations. *J. Softw. Eng. Appl.* 7:498–505. <https://doi.org/10.4236/jsea.2014.76046>
- Lenth RV. Emmeans: Estimated Marginal Means, aka Least-Square Means. R package version 1.8.6. 2023. <https://CRAN.R-project.org/package=emmeans>.
- Li X, Preisser EL, Boyle KJ, et al. 2014. Potential social and economic impacts of the hemlock woolly adelgid in southern New England. *Southeast. Nat.* 13:130–146. <https://doi.org/10.1656/058.013.s609>
- Li X, Boyle KJ, Preisser EL, et al. 2022. Property value effects of the hemlock woolly adelgid infestation in New England, U.S.A. *Ecol. Econ.* 194:107354. 14 pages. <https://doi.org/10.1016/j.ecolecon.2022.107354>
- Lindgren BS. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). *Can. Entomol.* 115:299–302. <https://doi.org/10.4039/ent115299-3>
- Liu F, Bittner TD, Whitmore MC. 2024. Environmental DNA assays for *Laricobius* beetles (Coleoptera: Derodontidae), biocontrol agents of the hemlock woolly adelgid in North America. *J. Econ. Entomol.* 117:1537–1544. <https://doi.org/10.1093/jee/toae116>
- Loo J, Ives N. 2003. The Acadian Forest: historical condition and human impacts. *For. Chron* 79:1–13. <https://doi.org/10.5558/tfc79462-3>
- MacQuarrie CJK, Derry V, Gray M, et al. 2024. Effect of a severe cold spell on overwintering survival of an invasive forest insect pest. *Cur. Res. Insect Sci.* 5:1–7. <https://doi.org/10.1016/j.cris.2024.100077>
- MacQuarrie CJK, Gray M, Bullas-Appleton E, et al. 2025. (preprint). The distribution of the hemlock woolly adelgids in Canada. *Canadian Entomol.* 157:1–20. <https://doi.org/10.1101/2024.07.18.604084>
- Mayfield III AE, Bittner TD, Dietschler NJ, et al. 2023. Biological control of hemlock woolly adelgid in North America: History, status, and outlook. *Biol. Control* 185:105308. 24 pages. <https://doi.org/10.1016/j.biocontrol.2023.105308>
- McClure MS. 1977. Population dynamics of the red pine scale, *Matsucoccus resinosa* (Homoptera: Margarodidae): the influence of resinosis. *Environ. Entomol.* 6:789–795. <https://doi.org/10.1093/ee/6.6.789>
- McClure MS. 1987. Biology and control of hemlock woolly adelgid. The Connecticut Agricultural Experiment Station, New Haven. Bulletin 851. 9 pages.
- McClure MS. 1990. Role of wind, birds, deer, and humans in the dispersal of hemlock woolly adelgid (Homoptera: Adelgidae). *Environ. Entomol.* 19:36–43. <https://doi.org/10.1093/ee/19.1.36>
- McClure MS. 1991. Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. *Environ. Entomol.* 20:258–264. <https://doi.org/10.1093/ee/20.1.258>
- Miller CA, Kettela EG, McDougall GA. 1971. A sampling technique for overwintering spruce budworm and its applicability to population surveys. Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre. Information Report M-X-25. <https://ostnrcan-dostnrcan.canada.ca/entities/publication/ac5be4e9-6783-430f-b8d6-ae3510bb99ff>. (last accessed March 2025)
- Parker WC, Derry V, Elliott KA, et al. 2023. Applying three decades of research to mitigate the impacts of hemlock woolly adelgid on Ontario's forests. *Forest. Chron.* 99:205–225. <https://doi.org/10.5558/tfc2023-024>
- Preisser EL, Oren KLF, Hain FP. 2014. Hemlock woolly adelgid in the eastern United States: What have we Learned? *Southeast. Nat.* 13:1–15. <https://doi.org/10.1656/058.013.s604>
- R Core Team. 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>
- Reaser JK, Burgiel SW, Kirkey J, et al. 2020. The early detection of and rapid response (EDRR) to invasive species: a conceptual framework and federal capacities assessment. *Biol. Invasions* 22:1–9. <https://doi.org/10.1007/s10530-019-02156-w>
- Rohr JR, Mahan CG, Kim CE. 2009. Response of arthropod biodiversity to foundation species declines: The case of the eastern hemlock. *Forest Ecol. Manag.* 258:1503–1510. <https://doi.org/10.1016/j.foreco.2009.07.002>
- Russo NJ, Elphick CS, Havill NP, et al. 2018. Spring bird migration as a dispersal mechanism for the hemlock woolly adelgid. *Biol. Invasions* 21:1585–1599. <https://doi.org/10.1007/s10530-019-01918-w>
- Sanders M, Tardani R, Locher A, et al. 2023. Development of novel early detection technology for hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae). *J. Econ. Entomol.* 116:168–180. <https://doi.org/10.1093/jee/toac175>
- Snuffling R, Evans M, Perera A. 2003. Presettlement forest in southern Ontario: Ecosystems measured through a cultural prism. *Forest. Chron* 79:485–501. <https://doi.org/10.5558/tfc79485-3>

- Spaulding HL, Rieske LK. 2010. The aftermath of an invasion: Structure and composition of Central Appalachian hemlock forests following establishment of the hemlock woolly adelgid, *Adelges tsugae*. *Biol. Invasions* 12:3135–3143. <https://doi.org/10.1007/s10530-010-9704-0>
- Stodola KW, Linder ET, Cooper RJ. 2013. Indirect effects of an invasive exotic species on a long-distance migratory songbird. *Biol. Invasions* 15:1947–1959. <https://doi.org/10.1007/s10530-013-0423-1>
- Williams JP, Hanavan RP, Rock BN, et al. 2017. Low-level *Adelges tsugae* infestation detection in New England through partition modeling of Landsat data. *Remote Sens. Environ.* 190:13–25. <https://doi.org/10.1016/j.rse.2016.12.005>
- Witt JC, Webster CR, Froese RE, et al. 2012. Scale-dependent drivers of ungulate patch use along a temporal and spatial gradient of snow depth. *Can. J. Zool.* 90:972–983. <https://doi.org/10.1139/z2012-065>
- Yamasaki M, DeGraaf RM, Lanier JW. 2000. Wildlife habitat associations in eastern hemlock – Birds, smaller mammals, and forest carnivores. In McManus KA, Shields KS, and Souto D. *Proceedings: Symposium on sustainable management of hemlock ecosystems in eastern North America*. USDA Forest Service, Northern Research Station, GTE-NE-267.
- Zuur AF, Ieno EN, Walker NJ, et al. 2009. *Mixed effects models and extensions in ecology with R*. Springer. <https://doi.org/10.1007/978-0-387-87458-6>