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Sticky traps as an early detection tool for crawlers of *Adelges tsugae* (Hemiptera: Adelgidae)

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Abstract

We developed an approach using sticky trap arrays as an early detection tool for populations of first-instar nymphs of the hemlock woolly adelgid (*Adelges tsugae* Annand), a pest of hemlocks (*Tsuga* spp. [Pinaceae]) in North America. We considered the detection rate of at least one nymph from trapping arrays consisting of one to six sticky panels, where we varied both the surface area of each trap that we assessed and the length of the trapping duration. We also estimated the time needed to set up, service, and assess groups of traps and attempted to relate capture of nymphs on traps to incidence and abundance of *A. tsugae* in the canopy above the traps. Arrays consisting of two traps provided a detection rate of 75% when 87.5% of the surface area of each trap was assessed, a process that required 38 min per array. The probability of detecting nymphs on traps left in the field for 5–6 d was similar to that for traps left for 12 d. The number of nymphs trapped in an array predicted the probability of finding *A. tsugae* in the canopy but only when all six traps were fully assessed. To reliably detect incipient *A. tsugae* infestations, we recommend placing arrays of traps at 1 km intervals along the perimeter of a stand during peak activity of first-instar sistentes nymphs and servicing these arrays every 5–7 d.

Key words: hemlock, hemlock woolly adelgid, trapping, early detection

Since detection of its arrival in Richmond, VA, from Japan ca. 70 yr ago, the hemlock woolly adelgid (*Adelges tsugae* Annand) has been killing hemlocks (*Tsuga* spp.) in the eastern United States. Today, this adelgid occurs in 20 eastern U.S. states and the District of Columbia (Limbu et al. 2018). Discoveries of this adelgid have also occurred in Ontario, Canada (Fidgen et al. 2014), but these outbreaks have been eradicated (Fidgen et al. 2019a). In 2017, the Canadian Food Inspection Agency (CFIA 2017) reported the discovery of another outbreak, this time in Nova Scotia (Weymouth). As of 2018, this infestation has spread to five counties (Digby, Yarmouth, Shelburne, Annapolis, and Queens Counties) of southwestern Nova Scotia. In 2019, the Canadian Food Inspection Agency reported two incursions of *A. tsugae* in southern Ontario (North American Plant Protection Organisation 2019). There is a need for effective and sensitive sampling tools and techniques to facilitate early detection of this invasive alien species (Fidgen et al. 2019a).

In North America, *A. tsugae* undergoes two generations a year exclusively on hemlock with each generation having an egg, nymph (four instars), and adult stage. The first generation occurs in spring (e.g., typically May to June at our study sites near Ithaca, NY), producing wingless adult females, called progredientes, and winged adult females, called sexuparae. The overwintering generation (June to May) develops only wingless adult females called sistentes (McClure 1987; Fidgen et al. 2014). The first-instar nymphs, called "crawlers", and the sexuparae are the only mobile stages of *A. tsugae* (McClure 1987). Crawlers of both generations are morphologically identical but vary in their seasonal timing and in the type of foliage they settle upon (McClure 1987; Limbu et al. 2018). Crawlers that develop into progredientes and sexuparae settle at the base of a needle, mainly on twigs of 1-yr-old foliage and amongst their sistentes mothers in early spring. By early summer, sistentes crawlers hatch and establish on shoots (i.e., current-year foliage), when available. Before the crawlers become sessile, they can be dislodged from their natal tree by wind, animals, or rain and end up on new host trees, on nonhost plants, on the ground or in water (McClure 1990; Turner et al. 2011; Fidgen et al. 2015). Sessile nymphs become covered by strands of wax (i.e., wool) exuded from pores on their body and are referred to as "ovisacs". When mature, the sexuparae fly to spruce (*Picea* spp.) and their offspring reproduce sexually but, so far, this form of reproduction has not been observed in North America (McClure 1987; Limbu et al. 2018).

Sampling to detect low-density pest infestations on the landscape can be laborious, time consuming, and expensive (Venette et al. 2002; Turgeon et al. 2010; Berec et al. 2015). Therefore, well-designed sampling techniques should provide acceptable performance but be as cost efficient as possible (Youngman et al. 1996; Turgeon et al. 2016). Detection of low densities of *A. tsugae* is challenging because populations are likely to establish high in a tree's crown where they are not easily seen (Evans and Gregoire 2007; Joseph et al. 2011) and tools and techniques to sample or reach that part of the crown are few (e.g., Fidgen et al. 2016). This suggests that alternative techniques would be useful in the detection of *A. tsugae*. McClure (1990) and Fidgen et al. (2015) demonstrated that sticky traps placed below the canopy can intercept falling crawlers but both studies were performed in stands where *A. tsugae* densities were higher than those that would be targeted in detection surveys. Thus, the technique has not been widely adopted.

Herein, we describe a study where we deployed groups of sticky traps below the crowns of hemlock trees lightly infested with *A. tsugae* to trap its mobile forms. We then used a resampling approach to determine both the minimum number of traps required and the minimum surface area of each trap that would need to be examined to detect low densities of *A. tsugae*. We also quantified the amount of time required to set up and process groups of traps so as to quantify their efficiencies for a fixed level of detection of a crawler. Finally, the canopy over the traps was sampled to examine the relationship between the number of crawlers on traps and the incidence of insects in the trees. We discuss how our findings could assist managers in the early detection of new outbreaks of *A. tsugae* or the delimitation of existing ones in natural stands.

Materials and Methods

Study Sites

We carried out this study in 2016. First, we selected six hemlock stands near Ithaca, NY (Table 1). Stands consisted of semimature *Tsuga canadensis* (L.) Carr. (mean ± SE; stem diameter at 130 cm above ground [$D_{130\ cm}$] = 41.2 ± 2.6 cm; height = 26.0 ± 1.1 m) mixed with (in order of relative density): yellow birch (*Betula allegheniensis* Britt. [Betulaceae]); sugar maple (*Acer saccharum* Marsh. [Aceraceae]); beech (*Fagus grandifolia* Ehrh. [Fagaceae]); white/red oak (*Quercus* spp. [Fagaceae]); white pine (*Pinus strobus* L. [Pinaceae]); tulip poplar (*Liriodendron tulipifera* L. [Magnoliaceae]); white ash (*Fraxinus americana* L. [Oleaceae]); black cherry (*Prunus serotina* Ehrh. [Rosaceae]); and shagbark hickory (*Carya ovata* (Mill.) K. Koch [Juglandaceae]). We used twig sampling (Fidgen et al. 2019a) to estimate population levels of *A. tsugae* in the canopy in late April and early May. Briefly, we removed two 45-cm-long branch tips from each of 10 trees with a modified Gilmour Commercial Tree Pruner (Robert Bosch Tool Corp., Peoria, IL; maximum reach 8.5 m). Next, the number of 1-yr-old twigs with at least one live ovisac and the total number of twigs on each tip was counted. In spring, living sistentes ovisacs that survived winter were significantly larger in diameter than early and mid-instar adelgid nymphs in small ovisacs that were killed by cold winter temperatures. This size difference indicates the presence of late instar nymphs, adults, and possibly egg masses. We calculated the percentage of twigs with at least one live ovisac (Incidence) using the equation:

$$\text{Incidence} = (TW_O \div TW) \times 100$$

where TW_O is the number of twigs with at least one adelgid ovisac and TW is the total number of twigs on a branch tip.

Trap Design and Deployment

We used flat sticky traps similar in design and construction to those described by Fidgen et al. (2015) but reduced their size to 20 × 20 cm from 25 × 25 cm. We reduced the size because the larger traps were difficult to manipulate in the field and to examine under most dissecting microscopes. The traps were made from the same commercially available, light green, 4-mm-thick corrugated plastic sheets (Synergy Semiochemicals Corp., Burnaby, BC, Canada) used to make prism traps for the emerald ash borer (*Agrilus planipennis* Fairmaire [Coleoptera: Buprestidae]) in Canada (Grant et al. 2011).

Groups of traps (hereafter "arrays") were installed in areas of each stand where hemlock was dominant. In each stand, we set six traps in a triangle formation, with one trap placed at each vertex of the triangle and one trap placed equidistant between each vertex. Each trap was 15 m from its nearest neighbor and below the canopy where twig sampling had taken place. We deployed seven of these arrays: one each per stand except at Fall Creek where we set up two. To set up an array, we hammered a square wooden stake (2.5 × 2.5 × 180 cm) into the ground (ca. 50-cm deep) at each position of the triangular formation. Next, an aluminum nail was used to secure the sticky trap to the top of each stake.

Trapping began on 10 May and ended on 23 June (Table 1), coinciding with the availability of progredientes crawlers (Fidgen et al. 2015). We replaced traps with new ones at irregular intervals every 5–15 d. A trapping period was defined as the time interval between trap deployment and trap replacement. We placed each collected trap into a clear 7.6-liter Ziplock Hefty Jumbo Slider bag (S.C. Johnson & Son Inc., Racine, WI) that was stored at −15°C until assessed. For our subsequent analyses, we used each trapping period in a stand as an independent replicate.

Table 1. Location of stands of *Tsuga canadensis* near Ithaca, NY, where we studied detectability of first-instar nymphs of *Adelges tsugae* using sticky traps in spring 2016

Stand	Latitude	Longitude	Start trapping	End trapping	Trapping periods	Incidence (% twigs)
Ellis Hollow	42.441115°	−76.409292°	9 June	23 June	2	3.0
Fall Creek 1	42.454372°	−76.450588°	10 May	9 June	3	0.0
Fall Creek 2	42.454653°	−76.452253°	10 May	23 June	5	0.2
Spring Brook	42.502345°	−76.741465°	11 May	8 June	4	0.0
Stevenson Forest Preserve	42.410086°	−76.638729°	11 May	23 June	6	0.5
Skaneateles Lake	42.821307°	−76.329264°	11 May	7 June	4	0.0
Texas Hollow	42.414111°	−76.492422°	11 May	23 June	6	0.07

Incidence = percentage of *T. canadensis* twigs in a sample of two, 45-cm-long branch tips from each of 10 trees with one or more *A. tsugae* ovisacs. See text for further details.

Trap Assessments

We transferred traps to room temperature for ca. 15 min before assessing them. To facilitate assessment, we traced a square grid on each bag over the sticky side of the trap, resulting in 16 cells of 25 cm^2 each. Then, a dissecting microscope (64×) was used to count the number of crawlers in each cell. Because trap replacement occurred at irregular intervals, we standardized the counts by dividing the number of crawlers found per array by the duration of the trapping period.

Statistical Analysis

To examine for the effect of the number of traps in an array and the surface area assessed per trap on the probability of detecting an *A. tsugae* crawler (i.e., detectability), we used a resampling approach. Resampling saves costs as compared to laborious field sampling because sampling is simulated using a computer program, which allows for many sample replications (Legg et al. 2014). To do this, we first reduced our field data set to include only instances where the daily trap count was one or fewer first-instar nymphs as might occur during detection surveys. To resample the arrays, we developed our own procedure, which involved resampling without replacement until either a crawler was detected on a trap or the entire array had been assessed (Fidgen et al. 2019b). We resampled 2,000 times each of the 24 *A. tsugae*-positive arrays for each array size (one to six traps) and each trap size (1–16 cells, 25 cm^2 per cell), giving 4,608,000 observations. We averaged the probabilities

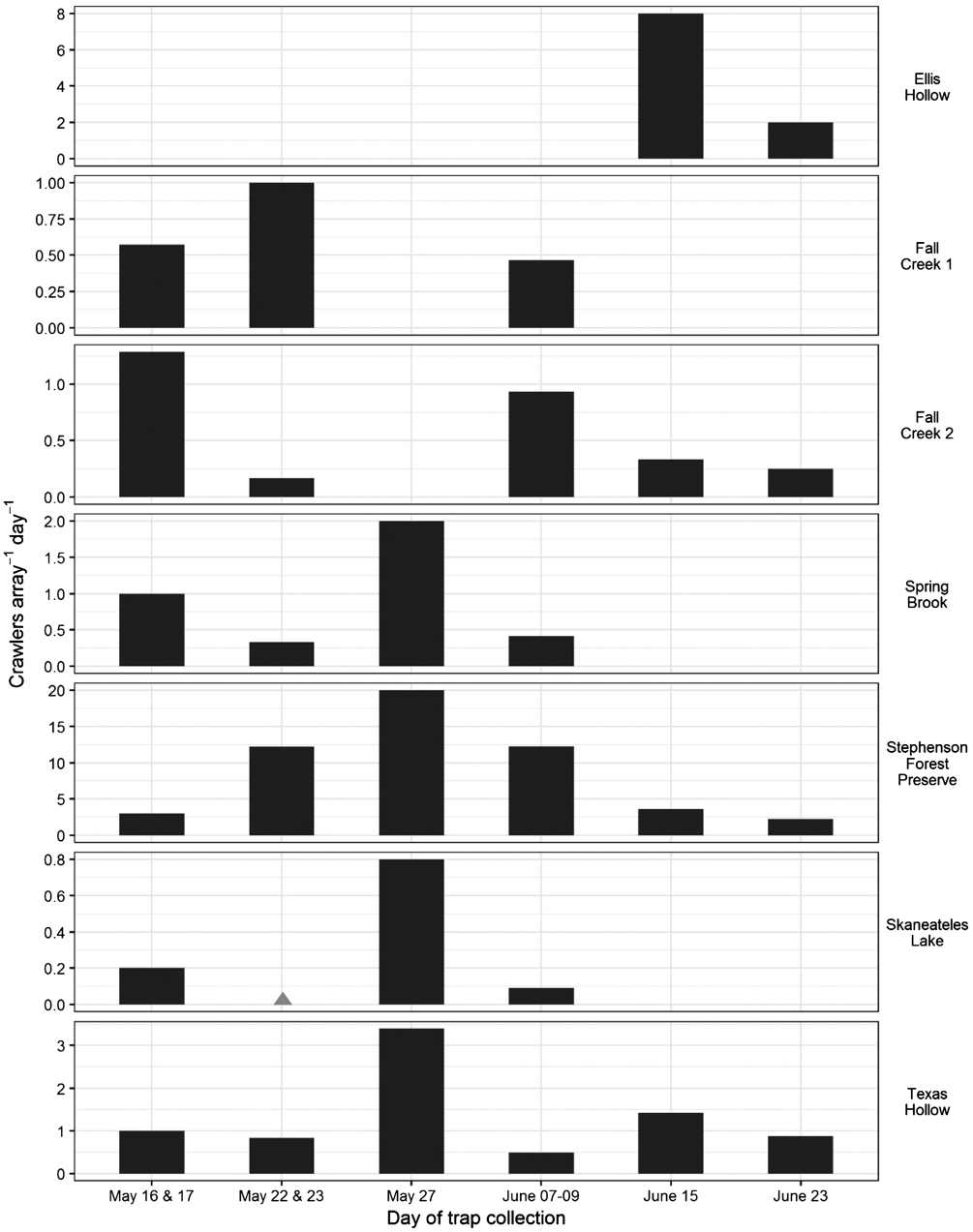

Fig. 1. Number of *Adelges tsugae* progredientes crawlers caught daily per array of six traps placed underneath *Tsuga canadensis* canopies in six stands near Ithaca, NY, in spring 2016. Two arrays were installed at the Fall Creek stand. See Table 1 for other details. Grey triangle indicates no crawlers were caught on traps; otherwise, no bar indicates traps were not set up.

for each array size with increasing surface area assessed per trap. The impact of minimizing the number of traps in an array and the surface area assessed per trap was examined for a fixed detectability.

We were concerned that the length of the trapping period might affect the probability of detecting crawlers because, in general, traps accumulate crawlers with time. Therefore, we compared detectability of a crawler for arrays with a trapping period of 12 d versus those lasting 5–6 d because these trapping periods were the most common ones used. The probabilities were compared using analysis of variance (ANOVA).

To see if the number of positive traps in the array and the daily number of crawlers caught on an array were related to the presence–absence of ovisacs in the hemlock canopy above an array, we fit separate logistic models with the number of positive traps in an array and the number of crawlers caught on an array as the predictor variables and the probability of detecting ovisacs in canopy as the response variable. Separate general linear models were fit using the same predictor variables and the incidence of ovisacs in the canopy as the response variable.

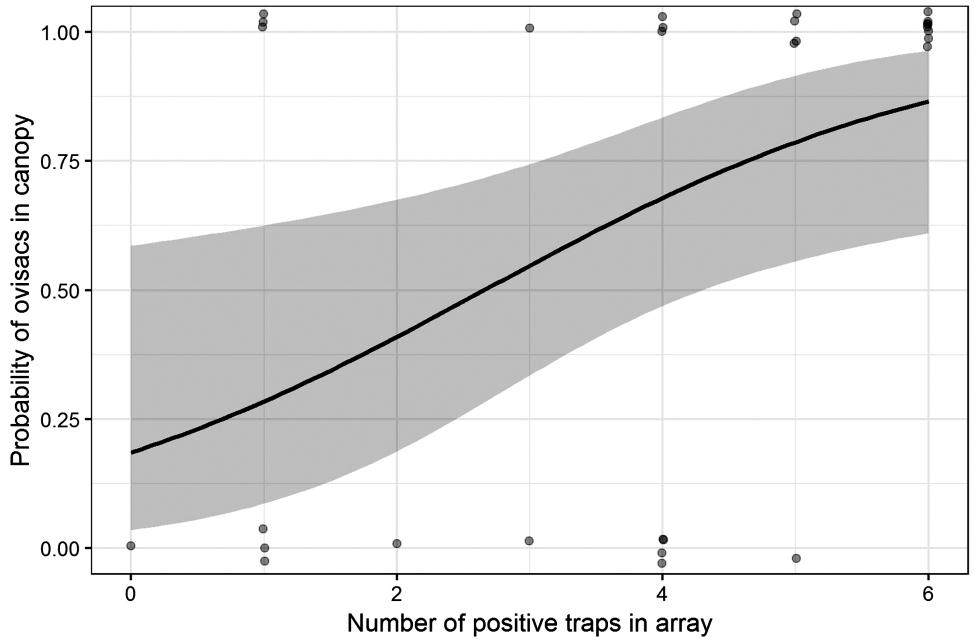

Fig. 2. Relationship between the number of positive traps for arrays of six traps and the probability (± SE) of detecting *Adelges tsugae* ovisacs in the *Tsuga canadensis* canopy above the array.

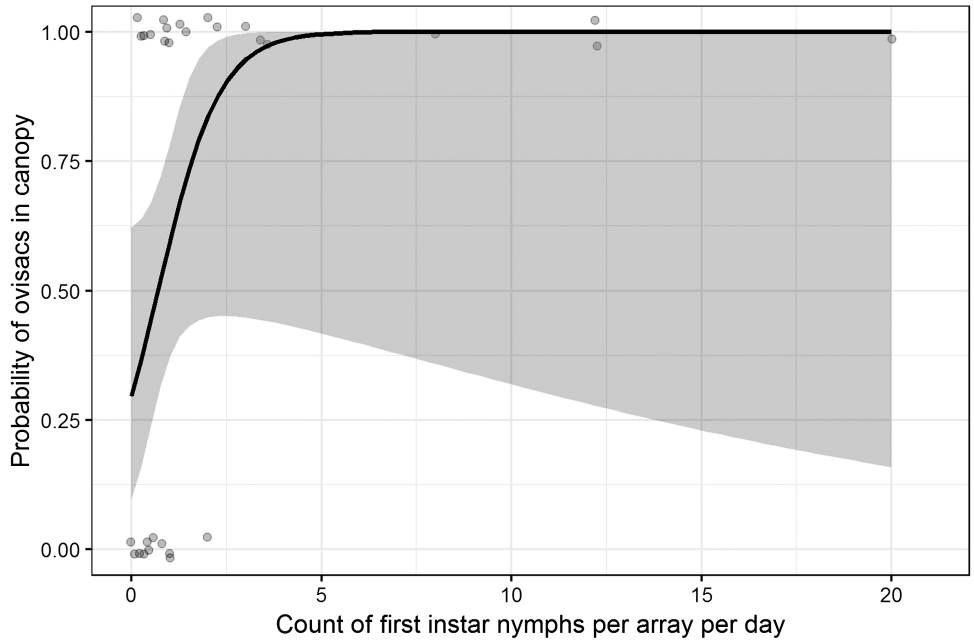

Fig. 3. Relationship between the daily counts of first-instar nymphs of *Adelges tsugae* found per six-trap array and the probability (± SE) of detecting ovisacs in the *Tsuga canadensis* canopy above the array.

All analyses were carried out in the R statistical computing environment version 3.4.2 (R Core Team 2017). Resampling was carried out using functions in the "dplyr" package (Wickham et al. 2018). General linear models, logistic models, and ANOVAs were fit using functions in the "stats" package (R Core Team 2017). Differences among levels of predictor variables were examined using functions in the "multcomp" package (Hothorn et al. 2008). Results were considered significant at $P \leq 0.05$ and are reported as mean ± 1 SE. To illustrate the tradeoff between the number of traps per array and surface area assessed per trap, we chose a detectability of 0.75 as that level is a recommended level for sampling pest populations (Karandinos 1976). All data and analysis code are available from the Dryad digital repository (Fidgen et al. 2019b).

Results

We found crawlers on all seven arrays. We completed 30 trapping periods for *A. tsugae* in the six stands during the experiment and caught crawlers in 29 of them (Table 1). There were two to six trapping periods per stand, with the trapping periods averaging 7.5 ± 0.5 d each. Crawler catch peaked the last week of May (Fig. 1). Crawlers were caught on 115 of the 180 traps we deployed with an average catch of 4.8 ± 0.7 crawlers per trap and 18.5 ± 5.9 crawlers per array. We caught 0.70 ± 0.09 crawlers per trap per day and 2.7 ± 0.8 crawlers per array per day. It took ca. 30 min (i.e., 5 min/trap) to set up an array of traps but only 15 min (i.e., 2.5 min/trap) to collect and replace traps. It took 13.3 ± 1.6 min to assess a negative trap but 6.1 ± 1.4 min to find the first crawler on a positive trap with eight or fewer crawlers. We also caught sexuparae on the traps but only at sites with a trapping period that ended on 23 June (Table 1).

We detected ovisacs in the canopy over four of the seven arrays when twig sampling (Table 1), finding an average incidence of 0.5 ± 0.4%. The probability of finding ovisacs in the canopy during twig sampling was related positively to the number of positive traps in an array (deviance, $D = 1.52$, df = 1, 28, $P = 0.005$; Fig. 2). Likewise, the probability of detecting ovisacs in the canopy was related positively

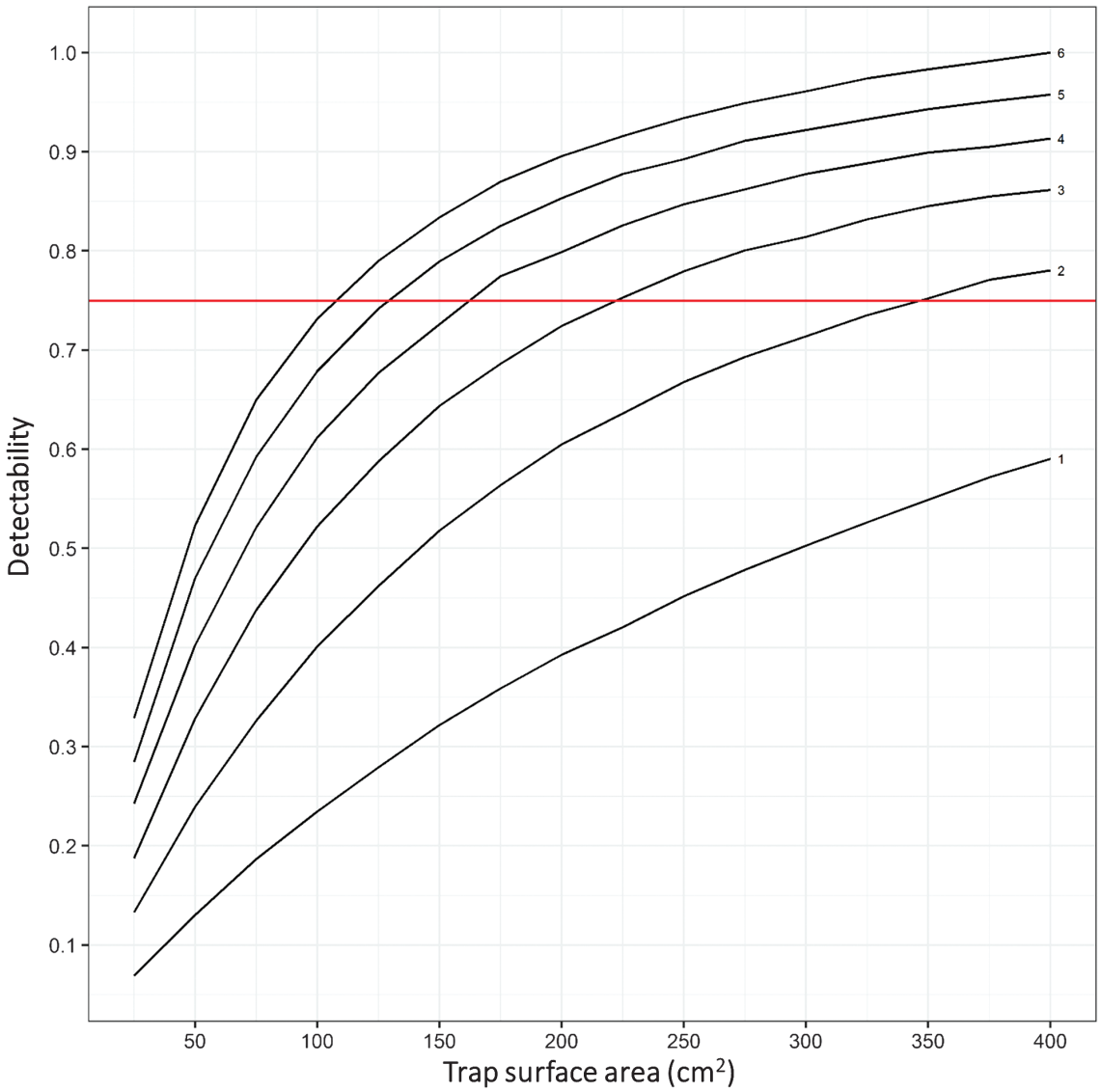

Fig. 4. Influence of surface area assessed per trap and number of traps in an array on detection of at least one first-instar nymph of *Adelges tsugae* during resampling simulations. The numbers at the end of each solid black line give the number of traps in each array; the horizontal line indicates a detectability of 0.75. Where lines intersect with horizontal line, it gives an optimal trap number—trap area assessed combination to achieve the desired detectability.

Table 2. Estimated time required to install, service, and assess arrays of sticky traps for first-instar *Adelges tsugae* nymphs based on optimal combinations of trap array size and area assessed per trap to achieve a detectability of 0.75

Array size (no. of traps)	Area (cm^2) of trap to assess	Time (min) required to:			Total time (min)
		Install array	Service array	Assess traps*	
2	350	10	5	23	38
3	225	15	8	27	50
4	175	20	10	30	60
5	150	25	12	25	62
6	125	30	15	25	70

*Based on an average of 0.825 min required to assess 25 cm^2.

to the daily crawler count in an array of six traps (D = 2.68; df = 1, 28; P = 0.02). We usually detected ovisacs in the canopy when three or more crawlers were intercepted in an array of six traps (Fig. 3). The incidence of ovisacs in the canopy over the array was not related to the count of crawlers on an array (D = 1.24; df = 1, 28; P = 0.13) or the number of positive traps in an array (D = 1.63; df = 1, 28; P = 0.23).

The reduced data set contained 24 arrays where we averaged one or fewer crawlers per trap. When we simulated the sampling of these arrays and traps, we found that detectability of an *A. tsugae* crawler increased when more traps and more surface area per trap were assessed (Fig. 4). To obtain a detectability of 0.75, we needed arrays of two or more traps. For this detectability, the time to set up, collect, and replace and scan traps for crawlers was shortest for arrays consisting of two traps (Table 2). To obtain a detectability >0.75 required arrays with a greater number of traps or required the assessment of more surface area per trap or both. For example, a detectability of 0.90 required an array of at least four full-sized traps. Lastly, there was no effect of duration of the trapping period on detectability (F = 0.001; df = 1, 11; P = 0.98).

Discussion

Our study is not the first one to use sticky traps to intercept *A. tsugae* crawlers (McClure 1990; Fidgen et al. 2015), but it is the first to develop a sensitive and efficient approach to detecting crawlers when adelgid incidence is low. We found that much smaller arrays than the six-trap array we tested can detect at least one *A. tsugae* crawler. For example, we show that we can obtain a 0.75 detectability (75% detection rate) using a two-trap array and examining 87.5% (350 cm^2) of each trap (Fig. 4). A three-trap array only increases the time cost by 12 min (Table 2), yet provides higher detectability (i.e., 0.85), if desired, when all of each trap is examined (Fig. 4).

One aspect needing further study is the placement of trap arrays in a hemlock stand. Low-density *A. tsugae* populations are typically aggregated (Gray et al. 1998; Fidgen et al. 2013) and colonizing *A. tsugae* populations are most likely to establish along stand edges (Costa and Onken 2006), presumably because the vectors that move *A. tsugae* to new stands are more active there (McClure 1990). Thus, placing several small arrays near stand edges may improve the odds of detecting *A. tsugae*. Another important aspect to consider is the number of arrays to deploy in a stand. This number will likely depend on many factors, including stand size, values at risk, desired detectability rate, the infestation size targeted for detection, and available resources (Turgeon et al. 2010; Berec et al. 2015). McClure (1990) evaluated wind-assisted crawler dispersal from a known infested stand and found that the maximum distance downwind of the stand where crawlers were intercepted was 1050 m. Thus, a good starting point for detecting crawlers in a stand might be to set up arrays ca. 1 km apart along the stand perimeter. The traps may also be useful to monitor *A. tsugae* population levels, particularly before and after the application of treatments to suppress populations. We advise that a grid of traps might accomplish such a task.

Deciding when and how often to sample are important considerations for operational sampling activities, particularly when the targeted life stage is available for a limited period of time (Venette et al. 2002; Berec et al. 2015). We sampled the progredientes (first generation) crawlers because the population of sistentes adults was low at our sites and we wanted to test the effectiveness of our sticky trap arrays when the incidence of adelgids was low. However, when attempting to detect *A. tsugae* in operational surveys, it would be advantageous to conduct trapping activities when the abundance of crawlers is highest, such as during the sistens crawler stage (McClure 1991; Gray et al. 1998). However, the timing of trapping for *A. tsugae* sistentes crawlers will vary geographically. Near Ithaca, trapping for sistentes crawlers could begin in mid-June, end in late July, and would have the added benefit of catching sexuparae (McClure 1990), which we detected on traps from the last trapping period (Fig. 1). The duration of the trapping period did not affect the detectability of crawlers, suggesting that traps can be left out for shorter periods of time without compromising detectability. This finding is significant as debris and nontarget arthropods build up on traps with time, potentially increasing the risk of missing crawlers amongst the debris and increasing the time to assess a trap. It may be possible to reduce the amount of debris and nontarget organisms intercepted on our traps if a mesh is placed over the traps (e.g., Sétamou et al. 2019). Our findings suggest that only one 5–7-d trapping period is needed, provided that it coincides with the predicted peak of crawler abundance, which could be predicted using a day-degree model (e.g., Salom et al. 2002).

The number of positive traps in an array predicted the probability of detecting ovisacs in the canopy (Fig. 2), as did the count of crawlers on an array (Fig. 3). Clearly, count of crawlers on traps was a better predictor of the probability of finding ovisacs in the canopy than the presence–absence of crawlers on traps. In contrast, the number of positive traps and the count of crawlers on an array were poor predictors of the incidence of ovisacs in the canopy. These models could be improved with additional information: 1) the effective sampling radius of an array—this feature is unknown but we suspect is likely large owing to natural dispersal of crawlers (e.g., 1050 m; see McClure 1990); 2) the number and area covered by trees that overtop the arrays—as this should correlate with the ability to detect and estimate populations of *A. tsugae* in the canopy; for example, at the Stephenson Forest Preserve stand, where we caught the highest number of crawlers per array but found few ovisacs above in the canopy, we found heavily infested trees 100 m to the north of our trap array; these trees likely contributed a high

number of crawlers to our array; and 3) the effectiveness of sampling tools used to sample the canopy. In our study, the pole pruners could only sample the bottom 8.5 m of trees measuring ca. 26 m in height. Therefore, most of the crown was inaccessible when using twig sampling. Indeed, all arrays caught crawlers but twig sampling only detected ovisacs in the canopy above four of seven arrays. This suggests that ovisacs were missed during twig sampling, either by not sampling enough trees, as discussed above, or not reaching high enough in the crown. The detection of ovisacs higher in the canopy could be improved using a sampling technique that reaches the upper crown, like ball sampling (Fidgen et al. 2019a), or other techniques that, for example, look for dislodged ovisacs on or near the ground-level (unpublished data). A more comprehensive assessment of *A. tsugae* populations in the canopy would be needed to assess the false-negative rate of our recommended trap arrays.

We have identified several features that can be used as a basis for a sampling protocol using sticky traps to intercept dislodged *A. tsugae* crawlers. We have identified an optimum surface area to assess per trap and an optimum number of traps to deploy per array. We have also identified a useful starting point for the placement and spacing of these arrays in hemlock stands. Lastly, we have recommended a desirable timing for trap deployment and the frequency of trap replacement to optimize detectability of crawlers. These findings provide valuable information on the development of an operational survey protocol using sticky traps as an area-wide detection tool when *A. tsugae* densities are low. Furthermore, our approach to resampling traps could be used to optimize the use of sticky traps for other minute insect pests.

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Data availability statement

Data from this study are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.qp60223 (Fidgen et al. 2019b).

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