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Development of Novel Early Detection Technology for Hemlock Woolly Adelgid, *Adelges tsugae* (Hemiptera: Adelgidae)

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Abstract

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, threatens hemlock forests throughout eastern North America. Management efforts focus on early detection of HWA to ensure rapid management responses to control and stop the spread of this pest. This study's goal was to identify an affordable, efficient trap to aid with airborne environmental DNA (eDNA) sampling approaches as an early monitoring tool for HWA. We initially compared HWA detection success between a standard sticky trap, commonly used for HWA monitoring, and trap designs potentially compatible with eDNA protocols (i.e., passive trap, funnel trap, and motorized trap). Passive, funnel, and motorized traps' estimated capture success probabilities compared to sticky traps were 0.87, 0.8, and 0.4, respectively. A secondary evaluation of a modified version of the motorized trap further assessed trap performance and determined the number of traps needed in a set area to efficiently detect HWA. By modifying the original motorized trap design, its estimated capture success probability increased to 0.67 compared to a sticky trap. Overall, the cumulative capture success over the 16-week sampling period for the motorized trap was 94% and 99% for the sticky trap. The number of traps did impact capture success, and trap elevation and distance to infested hemlocks influenced the number of adelgids captured per trap. As eDNA-based monitoring approaches continue to become incorporated into invasive species surveying, further refinement with these types of traps can be useful as an additional tool in the manager's toolbox.

Key words: early detection, environmental DNA, hemlock, hemlock woolly adelgid, monitoring

Hemlock trees are critical to both terrestrial and aquatic systems as they provide thermal cover, habitat diversity, and quality ecosystems for a variety of flora and fauna (Yamasaki et al. 2000, Snyder et al. 2002, Ford and Vose 2007, Toenies et al. 2018). Losing hemlocks can drastically alter the structure, composition, and function of ecosystems (Orwig and Foster 1998; Ellison et al. 2005, 2018). One of the leading causes of hemlock death and decline in eastern North America is hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, an invasive insect. Economic impacts of HWA in the United States have been estimated to be over $250 million per year, primarily from decreased property values and the cost of treating and restoring infested hemlocks (Aukema et al. 2011).

Hemlock woolly adelgids feed on hemlock nutrients and can kill trees in as little as four years (Havill et al. 2014). The adelgids cover themselves with a white, 'woolly' wax while feeding on the hemlocks, and these white masses, also known as ovisacs, are the visible part of an infestation on a tree. HWA completes two asexual generations, progrediens and sistens, repeating annually in its invasive range (Havill and Foottit 2007). Newly hatched adelgids are referred to as crawlers because they are the only mobile life stage and travel to settle at the base of hemlock needles to begin feeding on xylem parenchyma cells found in hemlock twigs. Progredientes emerge in spring and early summer as the largest crawler hatch of the year, and their summer-laid eggs hatch the sistens generation of crawlers that feed for a short time before entering a period of dormancy in late summer. In the late fall, sistentes come out of dormancy to feed and develop through the winter months until laying their eggs in the spring to continue the cycle (Havill and Foottit 2007). Birds, mammals, wind, and a variety of human activities (e.g., logging, planting nursery stock, and recreating) drive the dispersal and spread of HWA (McClure 1990), particularly during these crawler stages.

After the presence of the flocculent ovisac was first detected in the United States in the 1950's (Gouger 1971), HWA has spread throughout much of the northeastern United States with expansion westward into Michigan, where the current ongoing infestation was initially detected in 2015 (Michigan Department of Natural Resources 2021). Management efforts in Michigan are underway to control and stop the spread of HWA, and the main focus of management groups is on early detection. The primary method used in Michigan for detecting HWA is a visual assessment of hemlock branches, typically those within reach from the ground, for the presence of ovisac material. This is a considerable task for land managers given the estimated 170 million hemlock trees in the state. Visual assessments alone may not allow for the earliest detection of this insect if initial HWA infestations begin in the top part of the canopy (Evans and Gregoire 2007). These early infestations, as well as adelgid populations with low densities, may not be clearly visible on branches within reach of the ground and could give the false impression that HWA is not present in these areas (McClure 1990, Evans and Gregoire 2007). This lack of early detection could severely hinder rapid management responses that are essential for eradication efforts (Lodge et al. 2006).

Current HWA detection methods used by land managers include sticky traps (Fidgen et al. 2015, 2019), ball sampling (Fidgen et al. 2016, 2018), branch sampling (Costa and Onken 2006), remote sensing using GIS (Boucher et al. 2020), and ground surveillance (Costa and Onken 2006, CFIA 2018) (reviewed in Emilson and Stastny 2019). There can be many benefits to each of these methods, but some of the risks are that they can be labor intensive, consumptive of time and materials, nonspecific, and rely on moderate to severe infestations to discover HWA (Emilson and Stastny 2019). McClure (1990) and Fidgen et al. (2015, 2019) found sticky traps to be effective at catching adelgids in the crawler stage but identifying HWA individuals in nymph life stages can require at least some taxonomic expertise in areas where HWA is sympatric with other adelgid species (Limbu et al. 2018). Multiple disciplines have been successful in using genetic analysis of environmental DNA (eDNA), where DNA collected from the environment (i.e., soil, water, or air) is then genetically analyzed to determine if target species are present (Lodge et al. 2012, Giblot-Ducray et al. 2016). Given their promising findings in plant biology (Johnson et al. 2019, 2021a,b), airborne eDNA-based approaches may assist current monitoring efforts with a combination of trap collection followed by genetic analysis. Several studies have successfully applied eDNA-compatible traps in terrestrial settings to collect airborne samples to monitor species presence or absence of plants, fungi, and invertebrates, and this application includes invasive species detection (Folloni et al. 2012, Treguier et al. 2014, Quesada et al. 2018, Thomsen and Sigsgaard 2018, Valentin et al. 2018, Johnson et al. 2019, 2021a,b; Butterwort et al. 2022). Similar to how eDNA is being used in aquatic systems (for reviews see Yates et al. 2019, Rourke et al. 2022), the incorporation of quantitative polymerase chain reaction (qPCR) or amplicon sequencing (metabarcoding) with airborne eDNA approaches may also provide information on species presence and abundance estimates (Johnson et al. 2021b). Because wind can help facilitate the natural dispersion of HWA crawlers and may also displace ovisac material within a forest canopy (McClure 1990), the use of airborne eDNA-compatible traps for capturing individuals or HWA-related material may be an effective method to monitor for the presence of HWA.

Our goal for this study was to determine if an affordable, easy-to-use trap, that is compatible with eDNA approaches, would be able to capture airborne HWA material in a forest setting. We first conducted a preliminary study in 2020 in a high infested area to assess trap designs that potentially could be compatible with genetic analysis for HWA material and evaluate their effectiveness in capturing HWA. A secondary study in a low infested area was conducted in 2021 to identify the minimum number of traps that would be needed within a given area to maintain a high potential of detecting an HWA infestation. We also evaluated how capture success was influenced by a trap's distance to an infested hemlock tree and landscape features including elevation, slope, and aspect. Implementing this technology could help maintain effective management of HWA, reducing the overall time spent in the field for land managers.

Materials and Methods

Trap Design Testing

Trap Designs

To evaluate the effectiveness of various traps in capturing HWA for genetic analyses, we used four trap designs. The traps used in this study were: 1) motorized trap (Fig. 1A), 2) passive trap (Fig. 1B), 3) 8-funnel Lindgren funnel trap (Lindgren 1983; Fig. 1C), and 4) standard sticky trap (Fidgen et al. 2019; Fig. 1D). The motorized trap we used was a modification of a trap originally designed by Quesada et al. (2018) as a successful method for capturing airborne fungal spores in a forest setting with petroleum-jelly-coated microscope slides. We wanted to utilize an airborne eDNA trap that used petroleum jelly as a capture method because adelgids can be mobile. Although there has been previous success in capturing airborne eDNA with passive dust filters (Johnson et al. 2019, 2021a,b), we were concerned that type of trap model may not completely secure HWA crawlers once caught. Our motorized trap design included four petroleum-jelly-coated (Vaseline) microscope slides affixed to the trap by a perpendicular (plus-sign shaped) metal wire that attached to a battery-powered motor. The motor rotated the slides in a clockwise direction at approximately 30 RPM (In the Breeze, Bend, OR). Two slides were parallel (petroleum jelly facing upwards) and two were perpendicular (petroleum jelly facing outward) to the ground to collect any airborne material. An aluminum pie pan and plastic bag covered the motor to protect it from the elements. The passive traps were designed from a standing wind vane with all four petroleum-jelly-coated microscope slides attached to the wind cups with the petroleum-jelly coating facing upwards and slides parallel to the ground to capture airborne material; the slides rotated solely by the wind. Each microscope slide used in passive and motorized traps was 7.5 cm × 2.5 cm. The traps using petroleum-jelly-coated microscope slides (i.e., the motorized and passive traps) can be used for further genetic analysis, as multiple studies (Dvorak et al. 2015, Aguayo et al. 2018, Eaton et al. 2018, Quesada et al. 2018, Rojo et al. 2019) have outlined methods to successfully extract DNA from the petroleum jelly material.

The 8-funnel Lindgren funnel traps consisted of eight 20 cm diameter openings of each funnel for material to fall into with a collection cup at the bottom. We kept 45 ml of propylene glycol in the attached cup of the funnel trap for preservation of material. Lindgren funnel traps, originally designed for scolytid beetles, are commonly used to capture aerial insects (Lindgren 1983, Klimaszewski et al.

Fig. 1. Photos of each trap design used in this study: (A) motorized trap, (B) passive trap, (C) funnel trap, and (D) sticky trap.

2018) and have the potential to be compatible with downstream DNA analysis (Milián-García et al. 2021). However, the trap's use for specifically capturing HWA has not been evaluated previously.

The sticky traps used were similar to those used by Fidgen et al. (2019). To reduce the cost of materials, we assembled five sticky card insect traps on a 20 cm × 20 cm (400 cm^2) corrugated plastic board for each sticky trap. These traps have been a useful tool for monitoring HWA (Fidgen et al. 2015, 2019), and recent developments allow the potential for these traps to be compatible with genetic analysis of captured material (Butterwort et al. 2022).

Trap Deployment for Design Testing

The trap design testing took place at Pioneer Park (PIPK), Muskegon, Michigan, USA (Fig. 2; lat. 43.283323°, long. −86.364505°) a site with confirmed HWA infestations. Pioneer Park is 58.7 ha (145 ac) of county park and campground property along Lake Michigan. The public recreational areas are surrounded by forests dominated by eastern hemlock (*Tsuga canadensis*) with some mixed hardwood and other conifers, mainly white pine (*Pinus strobus*). We designated the HWA infestation level as high based on a sistens count assessment outlined by Evans and Gregoire (2007), (Sanders 2021). All traps were deployed in areas with known infested hemlock trees to test our trap designs.

All four trap designs (motorized, passive, funnel, and sticky traps) were deployed for four weeks in the month of July 2020, which is during the sistens crawler stage. We organized our experiment in a randomized block design with five blocks (Fig. 3). Each block comprised 36 cells for a total area of 625 m^2. One of each trap type was randomly assigned a location within every block using a random number generator. All traps were attached to standing poles 1.5 m from the ground. Trap contents were collected on a weekly basis for a total of four collection periods. Slides from the passive and motorized traps and the funnel trap contents were collected in sterile 50 ml vials and stored in a refrigerator (4°C). The sticky trap panels were collected in clear, plastic storage bags due to their large size, and stored in a freezer (−20°C).

Adelgid Capture Assessment Within and Between Blocks

We assessed differences in HWA capture success for each of the four trap designs within each block and evaluated HWA distribution between blocks to account for potential effects of spatial variation in HWA across the study site. To assess adelgid capture success of the motorized and passive traps, we examined the petroleum-jelly-coated microscope slides under a Nikon SMZ645 dissecting microscope and counted the total number of HWA crawlers from the four slides of each trap. To assess adelgid capture success for the funnel traps, we counted crawlers in funnel traps by placing each trap's contents into an individual petri dish and examining the contents underneath a dissecting microscope. To obtain adelgid counts for the sticky traps, we counted adelgids on each sticky trap using methods previously described by Dreistadt et al. (1998). Adelgids were counted on a 2.5-cm-wide vertical column down the center of each sticky insect card using a dissecting microscope. We used this technique on each of the five cards that made up every sticky trap.

To determine if spatial variation in HWA prevalence across our sampling site might impact our capture results, we evaluated HWA presence within each designated block at Pioneer Park (Fig. 3) by counting the number of ovisacs on hemlock branches using a method from the Pennsylvania Department of Conservation and Natural Resources (Johnson 2020). This was quantified at the block level since differing amounts of HWA between blocks could impact trap success in catching HWA. We randomly selected 10 trees within every block and numbered the lower crown branches within 7.5 m of the ground starting on the north side and moving clockwise around the tree. We used a random number generator to select five branches around each tree and counted the number of ovisacs within a 25 cm length of the distal part of each branch.

Statistical Analysis of Trap Design Efficiency

All analyses were conducted using the program R v 4.0.3 (R Core Team 2020). HWA estimates within each block and adelgid capture assessment data were non-normal despite transformations, thus we chose nonparametric analyses. To determine whether there were differences in HWA prevalence between blocks, we assessed differences between the average number of ovisacs counted from each block with a Kruskal–Wallis test using the package stats v 3.6.2. We estimated the probability that a nonsticky trap would capture HWA when a corresponding sticky trap (same block and same collection date) also captured HWA with a Wilson score interval (Wilson 1927) using the package binom v 1.1-1. We also assessed differences in capture success between the different trap types using a generalized linear mixed model (GLMM), with trap type as the fixed effect and block as a random effect; the sticky trap was used as the reference. This was performed in the R package lme4 v 1.1-27.1 (Bates et al. 2015). Tukey's post-hoc test was performed with the package multcomp v 1.4-20 (Hothorn et al. 2008) to evaluate differences in capture success across trap types. All statistical analyses used an alpha value of 0.05 to determine statistical differences.

We used results from this analysis, in part, to identify factors limiting trap success and measures to improve them. We modified select designs to improve capture success and tested how our alterations to the motorized trap improved capture success compared to our initial trap design.

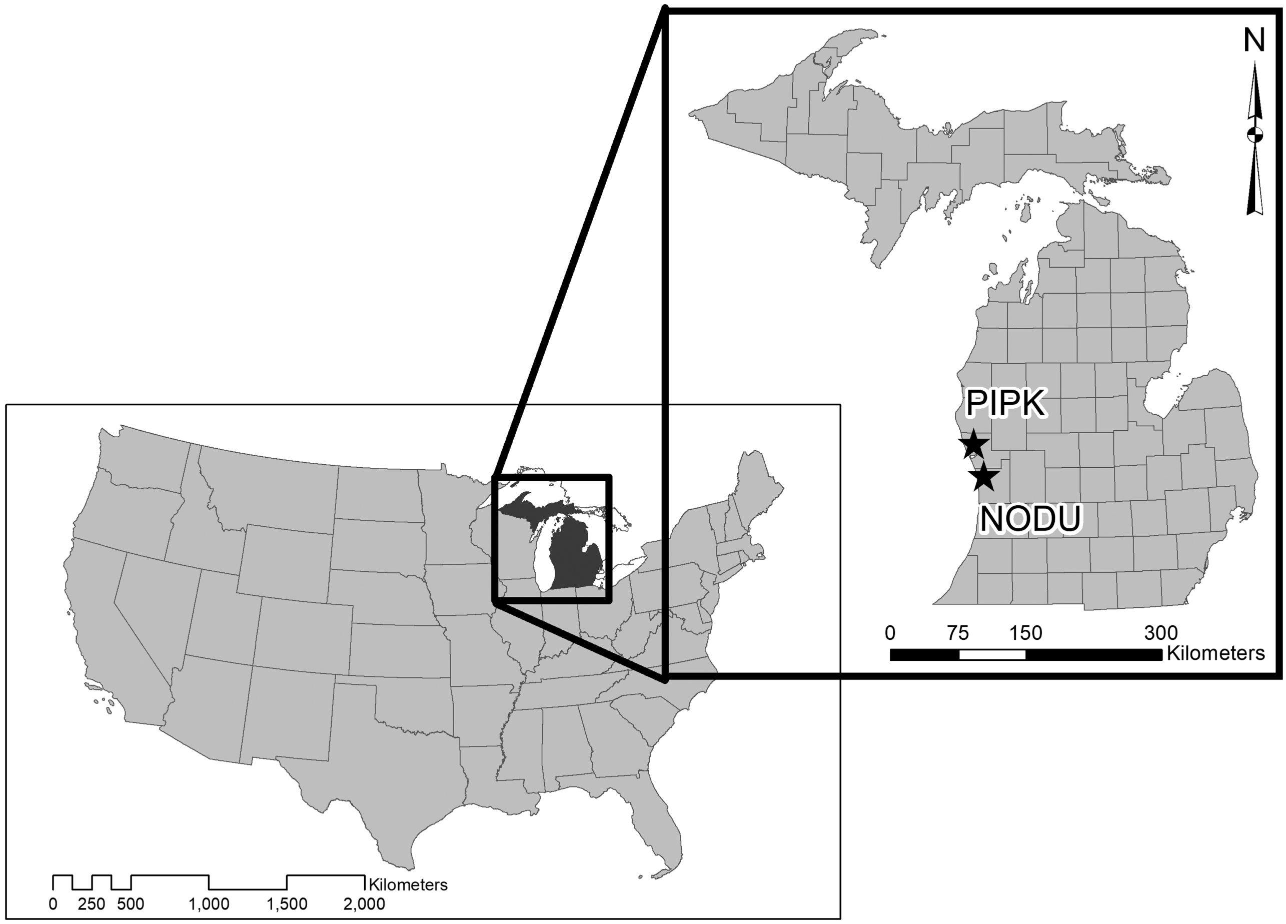

Fig. 2. Map of study sites: Pioneer Park (PIPK), Muskegon, Michigan, USA, and North Ottawa Dunes (NODU), Spring Lake, Michigan, USA, each denoted with a black star.

Evaluation of Capture Success Related to Number of Traps and Landscape Features

Given the durability of the motorized trap compared to the passive trap and its ease of use with potential downstream DNA analyses (see Discussion), we conducted further analysis to evaluate the number of traps that should be deployed in a given area to achieve a high probability of HWA detection. We also examined whether we could detect a relationship between the number of adelgids collected on a trap and the distance to an HWA-infested hemlock tree and general landscape features such as elevation, slope, and aspect.

The second part of our study took place at North Ottawa Dunes (Fig. 2; lat. 43.090484°, long. −86.247998°), a 240.2-ha (593-ac) Ottawa County Parks property of wooded sand dunes bordering Lake Michigan. The site consists of northern hardwood forest interspersed with eastern hemlock trees and other conifers. This is a site with a known HWA infestation, and we designated the infestation level as low based on a sistens count assessment outlined by Evans and Gregoire (2007), (Sanders 2021). We obtained Ottawa County Parks survey data (January–October 2020) with GPS locations of all hemlock trees within the park, as well as the locations of hemlock trees where visual surveys previously detected the presence of HWA ovisacs. We conducted our study in the southern part of the park where the largest clusters of HWA-infested hemlocks were located, and our entire survey range included areas both with and without hemlock trees.

For the trap efficiency assessment, we deployed a modified version of the previous motorized trap (Fig. 4) and sticky traps. While the motorized trap from the initial trap design study resulted in the lowest capture rate (see Results), we made significant modifications to this design that we felt corrected many of the flaws limiting its capture success. This included modifying the aluminum pan size to prevent the slides from being covered and arranging all petroleum-jelly-coated slides so that they were parallel to the ground (i.e., facing upwards). The base of the trap was changed by putting a circle (cut from corrugated plastic board) over the top of the perpendicular metal piece the slides were previously attached to. We then clipped the slides directly to the plastic circle, which gave each glass slide a more secure and even surface to lay flat when attached to the base. This helped prevent slide breakage, and it made collection and redeployment easier and faster for the user. We also slightly extended the distance that the slides hung from the motor to better prevent petroleum jelly from being wiped away when the wind blew the slides upward and they contacted the motor. The same 20 cm × 20 cm sticky trap design applied in our previous study was used in this experiment as a baseline comparison between the motorized trap design and a standard trap design commonly used for HWA detection.

Within North Ottawa Dunes, we established a 36.5-ha (90-ac) circle over our study area and sectioned it into 30 equal parts (Fig. 5). The 30 equal sections (3 acres each) were divided into five replicate groups (A–E), with six sections per group. Each of these six sections hosted a different number of paired motorized and sticky traps. Section one contained one pair of motorized and sticky traps, section two contained two pairs of traps, so on and so forth up to the sixth section containing six trap pairs. This resulted in a total of 105 motorized and 105 sticky traps for the entire 36.5-ha (90-ac)

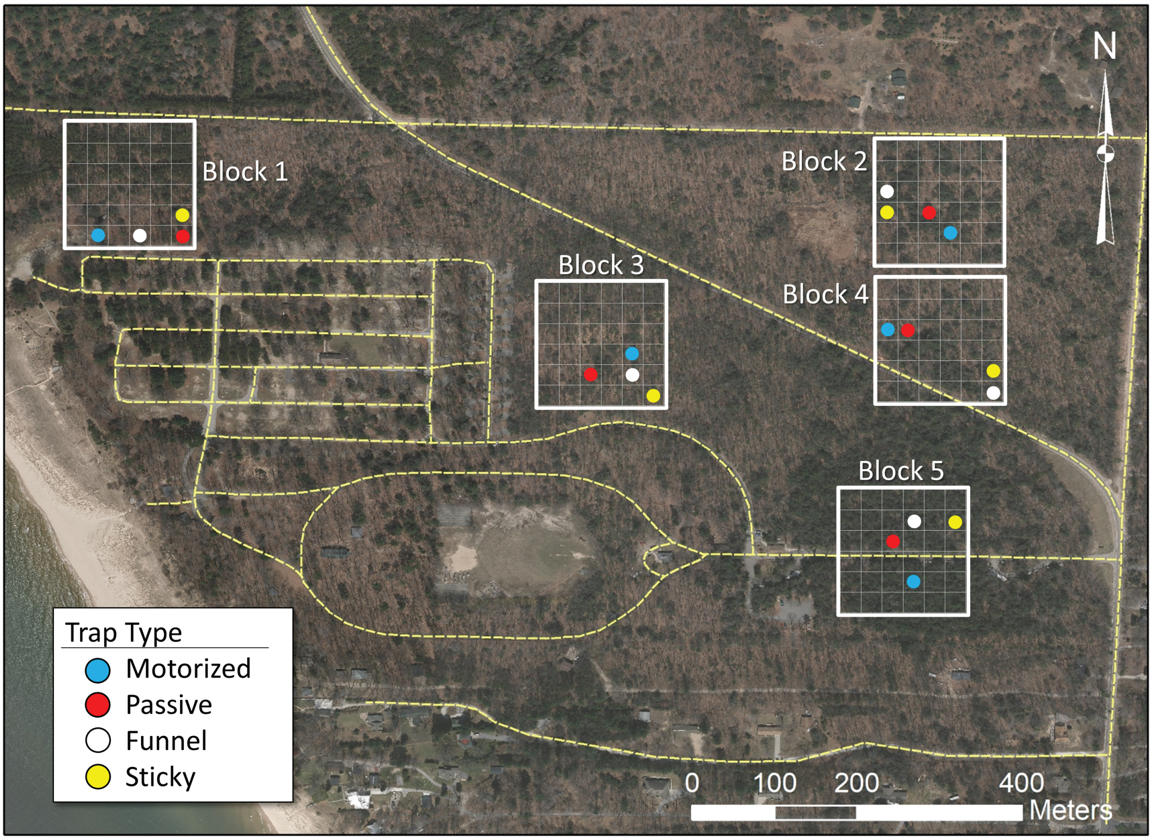

Fig. 3. Map of Pioneer Park, Muskegon, MI, USA, showing our randomized block design. A dot indicates a trap location within the block.

area, and the density of the traps within each section ranged from 1 trap per 0.2 ha (0.5 ac) to 1 trap per 1.2 ha (3 ac). In every replicate group, the number of trap pairs and trap placement within each section was randomly assigned. Traps were attached to a 1.5 m pole, and the motorized and sticky traps were placed 2 m apart at each trap location. Traps were deployed for 16 weeks from April 7 through 28 July 2021, during both annual HWA egg hatching events. Petroleum-jelly-coated slides from the motorized traps were collected biweekly and placed in 50 ml vials, and sticky traps were collected biweekly in clear, plastic storage bags. Trap samples were stored at room temperature until adelgids could be counted.

Adelgid Capture Assessment

After each biweekly collection, we counted the number of adelgids observed on each trap. For the motorized traps, the number of adelgids present on the four petroleum-jelly-coated slides was observed using a Nikon SMZ645 dissecting microscope, counted, and recorded. We assessed the number of adelgids collected on each sticky trap using the same method previously described for our trap design assessment (Dreistadt et al. 1998). For both the motorized and sticky traps, 20% of traps per collection period were recounted for quality assurance ($R^2 = 0.99$). When counting was completed for the motorized trap samples, we used dish soap to clean all microscope slides and 50 ml vials used for sample collection. These slides and vials were reused for other trap deployment and sample collection events throughout the trap assessment study.

Inverse Distance Weighted Spatial Interpolation Mapping

We created maps predicting distribution of HWA with the count data for each motorized trap by means of the inverse distance weighted (IDW) spatial interpolation method using ArcMap v 10.4.1 (ESRI 2016) to visualize how adelgid counts varied in our study area throughout the summer. The IDW method predicts likely HWA numbers based on a linear-weighted combination of count data for sample locations. This method is appropriate for clustered data. IDW predicts values for unsampled locations by assuming those values are related more to closer data points than to those that are farther away. We used a power of 2 and a nearest neighborhood search of 8 points in the analysis, so more localized trap counts influenced predictions of the nearby unsampled locations and to account for all cardinal directions surrounding a location.

Statistical Assessment of Motorized Trap Capture Efficiency

All statistical analyses performed in R used v 4.0.3 (R Core Team 2020). We estimated the probability that a motorized trap would detect HWA when the corresponding sticky trap detected HWA with a Wilson score interval (Wilson 1927) using the package binom v 1.1-1 to evaluate how our modifications to the motorized trap improved capture success compared to our initial trap design. We also used a GLMM to evaluate if the number of capture successes and failures differed between the sticky and motorized traps where trap type was considered a fixed effect, and the collection week and group ID (A–E) were included as random effects. This was performed in the R package lme4 v 1.1-27.1 (Bates et al. 2015).

To assess the level of spatial autocorrelation in the number of adelgids captured across our traps, we calculated Moran's I using the program GeoDa (https://geodacenter.github.io/faq.html). Euclidean distances were calculated between each trap point. The bandwidth was set to 0.001 so that the median number of neighbors for each point (i.e., trap) was five (min neighbors = 1; max neighbors = 8). We performed the same analysis for each two-week collection period

Fig. 4. Photo of the modified motorized trap used in our capture efficiency assessment.

when crawlers were present to test for significant spatial autocorrelation with 999 permutations.

We used a GLMM to evaluate if capture success within a 1.2 ha (3 ac) section was correlated with the number of traps within each section. This analysis focused on data collected from April 21 to July 28, when adelgid crawlers were present. In the full model, the fixed effect included the number of traps per section. The collection week and replicate group ID (groups A–E) were included as random effects; sections with one trap were used as the reference. The null model included the random effects collection date and group ID (A–E). We then used an ANOVA to determine if the addition of the fixed effect significantly improved the model. This analysis was run using the lme4 package v 1.1-27.1 (Bates et al. 2015). We used the R package multcomp v 1.4-20 (Hothorn et al. 2008) for post-hoc analyses to evaluate significant differences in capture success between each number of traps per section using a Tukey's post-hoc test. We also used a generalized linear model (GLM) to predict the number of traps that should be deployed within the 1.2-ha (3-ac) section to have a catch probability of 0.9 or greater. This analysis was performed for the active crawler period (April 21–July 28) and again with a subset of that data that represented the peak crawler period (May 19–June 16).

We assessed if trap elevation, slope, aspect, and Euclidean distance to the nearest HWA-infested hemlock impacted the number of adelgids caught in a motorized trap. The adelgid count data were non-normal and over-dispersed. Because of this, we used a GLM with a negative binomial distribution using the package MASS v 7.3-53.1. The full model consisted of adelgid counts as the dependent variable and Euclidean distance, elevation, slope, and aspect as the independent variables. A reduced GLM model was also run after removing the non-significant terms, and the optimal model was selected using the lowest Akaike's Information Criterion (AIC). All analyses used an alpha value of 0.05 to determine statistical differences. All data from both the 2020 and 2021 studies have been deposited into the Dryad repository: doi:10.5061/dryad.gb5mkkwt0.

Results

Trap Design Testing

With the Wilson score interval, we used the sticky trap as a reference point because if a sticky trap captured an adelgid, we would expect a corresponding trap in the same block to also capture an adelgid. If a nonsticky trap detected HWA every time a corresponding sticky trap did, then the estimated success probability would be 1. However, this does not indicate that sticky traps captured adelgids at every sampling period. Compared to sticky traps, the passive trap's estimated success probability averaged to 0.87 (95% CI = 0.62, 0.96), the funnel trap had an average success probability of 0.8 (95% CI = 0.55, 0.93), and the motorized trap averaged a 0.4 success probability (95% CI = 0.2, 0.64). There were no significant differences in the proportion of successful captures between the sticky, passive, and funnel traps. There was a significant difference in capture success between the motorized and sticky trap ($z = -2.78$, $p = 0.006$). All traps had some failures (where no adelgids were captured) across sampling periods and blocks. For HWA ovisac estimates within each block at PIPK, we accepted the null hypothesis that median values in ovisac counts were similar between blocks (Kruskal–Wallis test = 1.625, df = 4, $p = 0.804$). Thus, the HWA distribution was assumed to be similar across each block and should not have impacted the capture success of our traps.

Evaluation of Capture Success Related to Number of Traps and Landscape Features

Factors including trap durability, trap cost, sustainability in reuse of materials, general ease of use, and compatibility with eDNA sampling approaches (see Discussion) led us to pursue the use of the motorized trap for further long-term assessment in 2021. As stated previously, we made significant modifications that improved the overall success of this trap design. To evaluate how our alterations to the motorized trap improved capture success compared to our initial trap design, a Wilson score interval determined the modified motorized traps had an estimated success probability of 0.67 (95% CI = 0.62, 0.71) for capturing adelgids when its paired sticky trap also caught an adelgid. In total, the sticky traps had 487 individual capture successes and 338 capture failures; while the motorized traps had 396 capture successes and 442 capture failures ($z = 5.81$, $p = 6.45 \times 10^{-9}$). When evaluating the cumulative success of each trap over the course of the collection period, 104 of the 105 (99%) sticky traps placed in the 90-acre (36.5-ha) area captured at least one adelgid over the 16-week period, and 99 of the 105 (94%) motorized traps were successful over the 16-week period.

Based on Moran I's, only two collection periods displayed significant spatial autocorrelation with the number of adelgids captured, collection week two and collection week six (Collection week 2: Moran's I = 0.384, $z = 5.88$, $p = 0.001$; Collection week 3: Moran's I = 0.078, $z = 1.69$, $p = 0.06$; Collection week 4: Moran's I = −0.003, $z = 0.25$, $p = 0.19$; Collection week 5: Moran's I = 0.063, $z = -1.13$, $p = 0.12$; Collection week 6: Moran's I = 0.11, $z = 2.22$, $p = 0.03$;

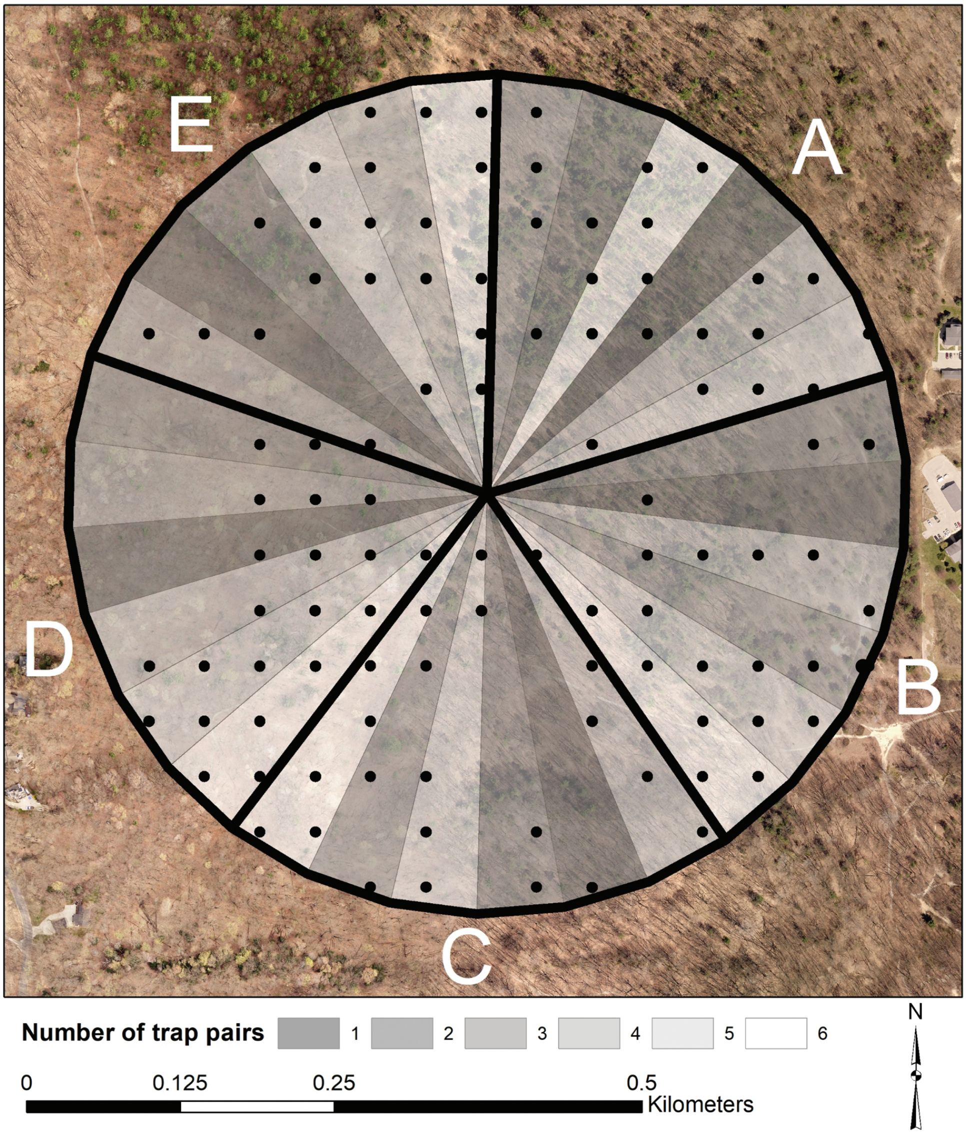

Fig. 5. Map showing our motorized trap capture efficiency assessment experimental design with 30 equal sections of a 36.5-ha (90-ac) circular survey area divided into five replicate groups (A–E) with six sections per group. Each section randomly was assigned between 1 and 6 pairs of motorized and sticky traps denoted on the map by a greyscale and black dots showing trap locations.

Collection week 7: Moran's I = 0.032, z = 0.71, p = 0.16; Collection week 8: Moran's I = 0.04, z = 1.05, p = 0.08). These periods are at the initial start of the progrediens crawler season and the end of the peak period.

The number of motorized traps included in each section significantly influenced whether traps within a section succeeded in capturing an adelgid (null model AIC: 207.7; full model with number of traps: AIC = 187.0, χ^2 = 30.8, df = 5, p = 1.07 × 10^{-5}). Based on Tukey's multiple comparison, sections with four, five, and six traps were significantly more successful than sections with one trap within a 1.2-ha (3-ac) section (4 vs 1, z = 3.58, p = 0.004; 5 vs 1, z = 3.88, p = 0.001; 6 vs 1, z = 3.58, p = 0.004). We also used a GLM to evaluate the number of traps per 1.2-ha (3-ac) section that would be needed to have a 0.9 probability of capturing an adelgid. When we included the active crawler periods, five traps per section are needed (Fig. 6A). When we subset the data to only include periods of the peak progrediens crawler stage (May 19th–June 16th), the number of traps needed per section decreased to two traps (Fig. 6B).

Spatial distribution of adelgid capture success varied throughout the HWA crawler period when HWA is most mobile (Fig. 7A–G). Spatially interpolated values predicted the potential number of adelgids captured if traps were placed in areas between our trap locations. We found that as the HWA progrediens crawler stage progressed, we captured an increasing number of crawlers, and these numbers peaked on June 2nd. The number captured began to decrease on June 16th, and a smaller proportion of traps captured crawlers through the sistens generation by the end of the study period on July 28th. Between May 19 and June 16 (i.e., the peak HWA crawler stage of the progrediens generation), the interpolated values show that traps could be placed almost anywhere in the study area and have the potential to capture adelgids (Fig. 7B–D). For example, only 11–14.4% of the study area had interpolated values equal to zero crawlers. Outside of the peak crawler stage, the geographical area that is likely to not catch crawlers (interpolated values = 0) was larger. At the beginning of the crawler stage (mid-May; Fig. 7A) and when the number of

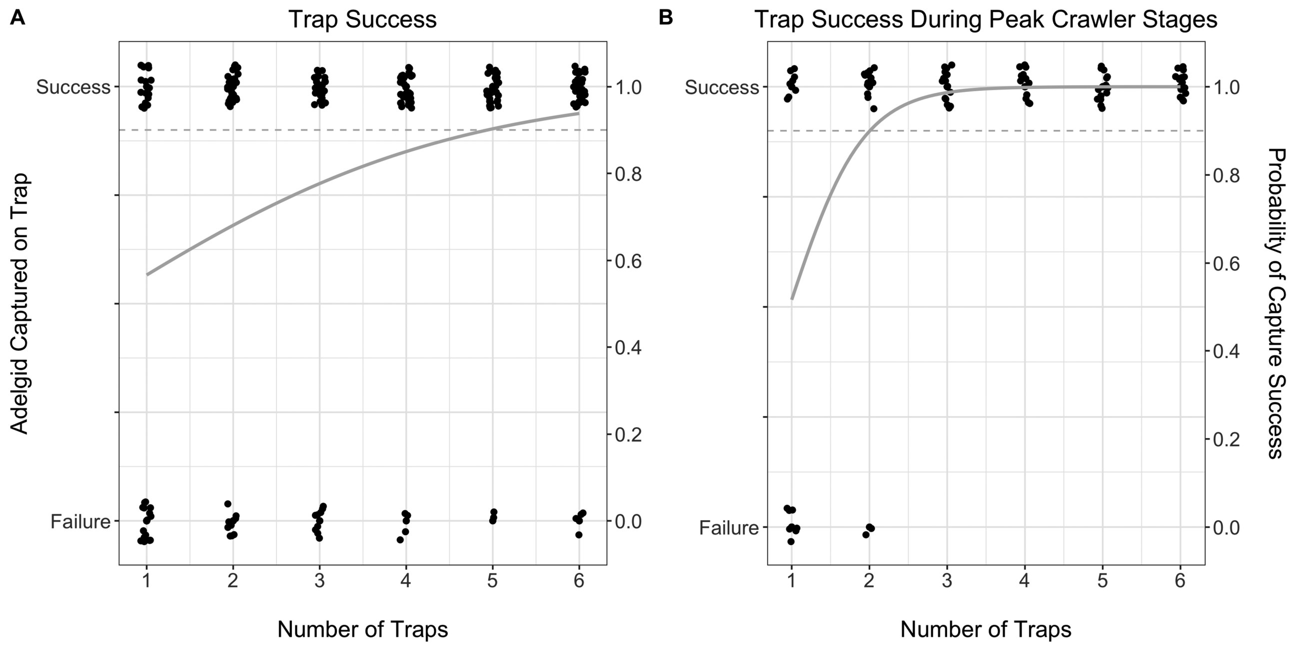

Fig. 6. Logistic regression estimating probability for the number of traps needed to capture HWA within a 3-acre area. (A) Logistic regression based on data collected from the active crawler season (April 21–July 28); (B) Logistic regression based on data collected from the peak crawler season (May 19–June 16). Each dot represents either a trap success (adelgid captured) or a trap failure (adelgid not captured) for each sampling period. The dashed grey line indicates the 0.9 detection probability.

crawlers started to decrease after the peak (late June; Fig. 7E), the geographical area predicted to catch zero crawlers was approximately 30%. In July, when crawlers are less active, the proportion of the study area predicted to catch zero crawlers increased to ≥50% (Fig. 7F and G). However, it should be noted that much of the area that was not likely to catch crawlers also tended to have a lower density of hemlock trees. These maps also showed a close association between the number of adelgids captured and where hemlocks previously identified as containing ovisac material (purple stars) were clustered. Traps near clusters of infested hemlock trees tended to have higher adelgid numbers, and this pattern was most obvious during the May 19th–June 16th sampling period (Fig. 7C and D).

Results also suggest that landscape features may influence HWA detection. In a full model with all explanatory variables, slope and aspect were not significant. However, a reduced model with slope and aspect removed lowered the AIC score and thus improved the prediction, suggesting that elevation and EucDist influenced HWA detection (Table 1). Therefore, we designated the reduced model as the optimal model. While the reduced model was slightly improved based on AIC values compared to the full model, it was not significantly better (ANOVA, $p = 0.08$).

Discussion

Our first goal of this study was to identify an affordable, durable, sustainable, easy-to-use trap that could effectively capture airborne HWA material, and would be easily compatible with eDNA approaches; some of these factors are summarized in Table 2. The passive trap design was most similar to the sticky trap in catch rates (0.87 success probability). However, these traps were the least durable of this study with broken traps noted at every collection in each block. Continual replacement of these traps could lead to increased time, effort, and cost by management teams, as well as lost data, if they were to be used for long-term monitoring. For traps that did survive between collecting periods, further processing for eDNA-compatible methods was efficient. Counting the adelgids took approximately 5–10 min. Once the adelgid counts were finished, all of the petroleum jelly material was scraped off the slides using a sterile spatula or sterile toothpick directly into 1.5 ml centrifuge tubes for further DNA extraction, and this process took 2–5 min. The ability to quickly sample all the material that has been captured on the trap for further DNA processing is one of the advantages that makes this type of trap attractive for eDNA-based approaches. If the durability of these traps were increased, they would be one of the most sustainable for eDNA methods. Once the slides are processed, they can be washed, and then either bleached and autoclaved or UV sterilized and reused for future deployment. Also, for individuals collecting multiple samples within a given period, redeployment costs are low even if new microscope slides are used.

The funnel trap had the second highest capture success (0.8 success probability) but was also the most expensive of the traps with an initial cost of approximately $100 (including the trap, shipping, and materials for deploying). Counting crawlers took longer, >30 min, since they contained more bycatch of nontarget species. While we did not try to extract DNA from these samples using an eDNA-based approach (where everything in the sample is extracted), this may be more cumbersome given the amount of bycatch we obtained. Depending on the amount and type of bycatch, which for us included species in Diptera, Coleoptera, and Lepidoptera, DNA extractions may need to be performed in larger volumes or require multiple DNA extractions per trap if using kit-based extractions; thus, increasing the overall cost of the eDNA-based approaches. It may be possible to filter samples to only include smaller specimens, but then we may miss any remnant DNA that might be present on larger HWA ovisac material or hemlock needles. These traps were highly sustainable given that the funnel traps can be used multiple times and the only redeployment cost would be the cost of refilling

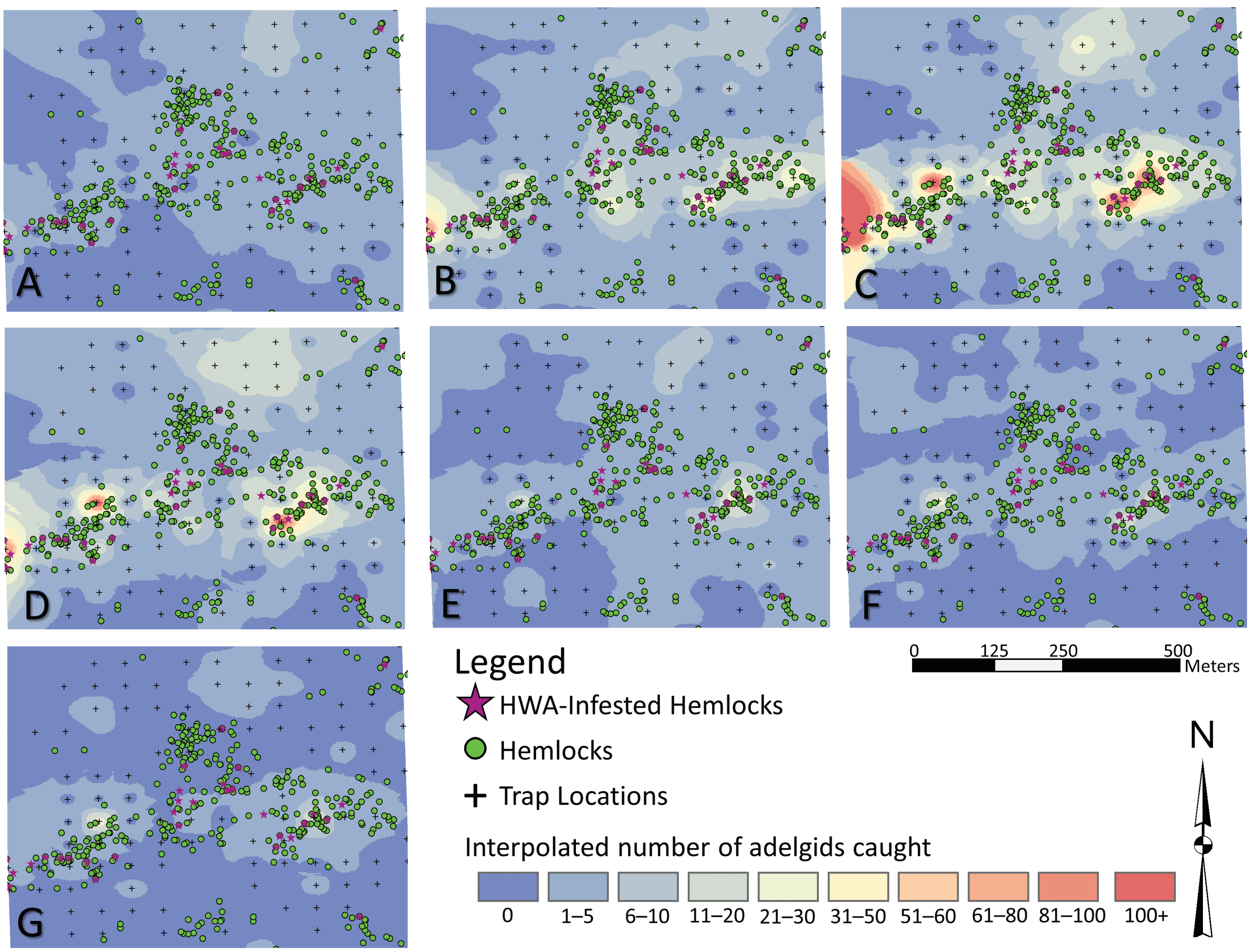

Fig. 7. Inverse distance weighted spatial interpolation maps created for every 2021 collection period with capture success using the count data of each motorized trap. Collection dates: (A) May 5, (B) May 19, (C) June 2, (D) June 16, (E) June 30, (F) July 14, and (G) July 28.

the collection cup with propylene glycol. However, given the size of these traps, appropriate sterilization between uses may be more problematic.

The motorized trap was the least successful in the 2020 study (0.4 success probability), but this success was increased with trap modifications in 2021 to 0.67 success probability when compared to paired sticky traps. We did evaluate this trap for a longer period in 2021 (April–July), and when assessed over the full 16-week period, the cumulative success (how many total traps caught an adelgid out of the 105 traps deployed) was 94% compared to 99% of the sticky traps. This trap was sturdier than the passive trap. Like the passive trap, the lack of bycatch decreased the time needed to count adelgids to 5–10 min, and these data may be important for initial quantitative assessment. All the petroleum jelly was completely removed from the slides and placed in a 1.5 ml centrifuge tube, resulting in limited sample loss from slide processing to DNA extraction. Within a future eDNA-approach framework, this is important if this method were to be incorporated into qPCR-based abundance estimates. Like the passive trap, the ease of sampling all the trap material for genetic analysis makes this a promising option for future eDNA sampling. In terms of sustainability, they are also like the passive traps, where slides can be easily cleaned, sterilized, and reused. However, the batteries of the motor component would need to be replaced over time, which leads to more maintenance for these traps.

The sticky trap is commonly used for HWA monitoring and has been shown to be highly effective in capturing HWA (McClure 1990, Fidgen et al. 2015, 2019). For our 2021 study, it performed better than the motorized trap for the individual two-week monitoring periods. However, there can be some drawbacks for this type of approach as well. Unlike the passive, motorized, and funnel traps, sticky traps are single-use traps and need to be replaced every collection period, increasing costs for long-term monitoring. Like the funnel traps, there was also more bycatch present compared to the passive or motorized trap. This increased the amount of time needed for adelgid counts to >30 min per trap. Proof of concept methods has been developed for using eDNA approaches with sticky traps (Butterwort et al. 2022); however, these have been done with larger insects and with mock insect communities. Given the amount of bycatch present from the traps and the large surface area of the traps (400 cm^2 for this study), the potential complications noted with the funnel traps for eDNA-based approaches may also apply to sticky traps. Primarily, DNA extractions may need to be performed in larger volumes or multiple extractions may need to be performed to sample the contents from the entire trap. This would potentially increase the cost and time required for DNA processing. However, we did not test this, and depending on the size of the trap, location of the trap, and time of collection, this may not be a significant issue.

Given the durability and compatibility of the motorized trap with eDNA-based methods, we further evaluated the efficiency of this trap (after trap modifications) and examined factors that may impact adelgid capture success. We first evaluated if the number of traps placed within a given area had a significant impact on whether adelgids would be captured. We found that there was a significant impact of the number of traps per section on adelgid catch success. When evaluating the dataset for the active crawler period, we found that five traps would be needed per 1.2-ha (3-ac) section to have a 0.9 probability of capturing an adelgid. Although, based on Tukey's multiple comparison, the only significant differences in capture success were between sections with four, five, and six traps compared to sections with only one trap. When evaluating trap success during only the peak crawler stage, when the number of adelgids is at its highest, the number of traps needed to reach a 0.9 probability of capturing an adelgid decreased to two traps per section. This difference is likely due to lower capture success in sections with fewer traps when the number of crawlers present were lower (i.e., early and late collection periods). Therefore, it would be recommended to have a higher density of traps if sampling during these periods or in areas where HWA has not been previously detected and infestation levels would likely be very low.

Table 1. Results of the full and reduced GLMs used to assess how landscape variables impacted adelgid numbers

Variables	Estimate	Standard error	*z*-value	*p*
		Full model		
(Intercept)	18.284	5.489	3.330	8.67×10^{-4}
Northeast	1.152	0.514	2.24	0.025
East	−0.66	0.565	−1.169	0.242
Southeast	0.395	0.538	0.735	0.462
South	0.045	0.584	0.077	0.938
Southwest	0.597	0.492	1.213	0.225
West	0.169	0.478	0.354	0.723
Northwest	0.672	0.466	1.442	0.149
Slope	0.006	0.046	0.134	0.893
Elevation	−0.077	0.029	−2.597	0.009
EucDist	−0.004	8.21×10^{-4}	−5.263	1.42×10^{-7}
(AIC = 832.4)				
		Reduced model		
(Intercept)	17.295	4.974	3.477	5.07×10^{-4}
Elevation	−0.069	0.027	−5.139	2.76×10^{-7}
EucDist	−0.004	8.04×10^{-4}	−2.624	0.009
(AIC = 830.31)				

The full model used trap elevation, slope, aspect, and Euclidean distance (EucDist) to the nearest HWA-infested hemlock tree as explanatory variables for adelgid number caught by a motorized trap. The reduced model used only trap elevation and EucDist as explanatory variables for the number of adelgids caught. For the aspect variable, North was considered the reference variable in the GLM.

The cumulative adelgid capture success for the motorized traps was 94% over the full 16-week period, and this ranged from 22% to 72% for each two-week collection interval. By comparison, the success rate of the sticky traps ranged from 26% to 86% for each collection period and the cumulative success was 99% for the 16-week period. These success rates closely followed trends of adelgid crawler prevalence based on the timing of each life stage (progrediens and sistens). During the July 15–July 28 collection period, a period when HWA sistens crawlers become less mobile as they settle on hemlock needles, the motorized traps had their lowest adelgid capture success rate (21.9%), and the sticky traps also had a lower success rate of 25.7%. The higher success of the sticky traps in comparison to the motorized traps is not surprising attributing to their much larger surface area (400 cm^2) compared to the four microscope slides used with a motorized trap (75 cm^2 total). Further modifications of traps using microscope slides to increase the catch surface area would be relatively easy by increasing the number of petroleum-jelly-dipped slides used or increasing the size of the slides.

In our assessment of how trap elevation, slope, aspect, and Euclidean distance to the nearest HWA-infested hemlocks impact adelgids captured for the motorized traps, we found that trap elevation and distance to infested hemlocks had more of an effect on the number of adelgids captured than slope or aspect. This makes sense as the data generally showed that traps closest to infested hemlocks caught the most adelgids throughout the study (Fig. 7), and traps at lower elevations typically caught more than those at the top of a dune. It is important to state that there could also be other variables outside of what our study evaluated that could explain variation in the number of adelgid captures across our study site. Fig. 7C and D shows a cluster of traps in the northeastern part of our survey area that captured many adelgids but are not as close to infested hemlocks as most of the other highly successful traps. This could be due to wind pushing adelgids to those traps, as a lot of northeasterly winds prevail from Lake Michigan in this area. Those northeastern traps are also downhill from the nearest infested trees, so this could help facilitate adelgid movement to them. There could even be a closer infested hemlock tree that we could not consider since Ottawa County Park's HWA survey data for this park ended October 2020, and our study took place summer 2021. Also, infestation level of each individual hemlock tree could play a role as a heavily infested tree would produce more adelgids than a tree with just a few individuals.

Table 2. Comparison of the different categories we assessed for each trap type (i.e., sample processing time, cost, sustainability, sturdiness, HWA eDNA-analysis compatibility) in addition to HWA capture success for the initial trap design testing in 2020

Trap type	Sample processing time	Trap cost	Redeployment cost	Sustainability rating	Sturdy	eDNA compatibility
Motorized	5–10 min	$15.00	$1.00[a]	Third	Yes	Easy
Passive	5–10 min	$20.00	$0.20[b]	First	No	Easy
Funnel	≥30 min	$100.00	$0.60[c]	Second	Yes	Moderate
Sticky	≥30 min	$10.00	$7.00[d]	Fourth	Yes	Moderate

We rated each trap type on sustainability with 'first' being considered the most sustainable. Redeployment costs were calculated from the use of brand-new materials needed to redeploy a trap for each collection period, and these costs were averaged for a single trap from the total cost of redeployment over the four weeks of the study. We also acknowledge cost of materials can vary by country and region.
All trap costs include materials, shipping, and hardware needed for deployment.
[a]Includes cost of additional batteries, if needed, and new petroleum jelly for slides.
[b]Includes the cost of new petroleum jelly for slides.
[c]Includes the cost to refill the collection cup with 45 ml of propylene glycol.
[d]Includes the cost of a new sticky trap.

Among the few studies to assess the use of traps in detecting HWA is McClure (1990) and Fidgen et al. (2015, 2019), both of which used sticky traps to catch adelgids in the mobile crawler stage. Like McClure (1990) and Fidgen et al. (2019), our study suggests that trap distance to HWA-infested hemlock trees and the number of traps deployed impact capture success. Many states, such as Michigan, primarily use visual assessments to find new HWA infestations, but these on-the-ground surveys can miss early invasions that may only be present in the top part of the trees' canopy (Evans and Gregoire 2007). The motorized traps we evaluated are not as efficient as the sticky traps for monitoring HWA on a short-term scale, but our results showed that the cumulative success of the motorized trap was 94% compared to 99% for the sticky traps. We were able to increase the capture efficiency of the motorized traps between the 2020 and 2021 designs, and further modifications for either the passive or motorized traps can be made to increase their short-term capture success rates and durability in the field. In our first experiment, our initial motorized trap design had a 20-cm diameter aluminum pan covering the top of the trap to help protect the motor from the elements, and this allowed the pan to cover the width of the microscope slides hanging below the motor. We also initially had two slides facing up (parallel to the ground) and two slides on their side (perpendicular to the ground), as originally outlined in Quesada et al. (2018). We thought having two slides perpendicular with the petroleum-jelly-coated side facing the direction the slide rotated in would help increase the chance of collecting airborne material with a motorized trap. However, our results showed this might not be the case for our target species since the parallel slides often had more crawlers on them compared to the perpendicular slides. In our second trap efficiency experiment with the motorized trap, we put all four slides parallel to the ground (face-up), and we reduced the size of the aluminum pan covering by half. We believe these modifications attributed the most to the motorized trap's higher success in 2021 compared to 2020.

Further refinement of these passive and motorized traps can increase their capture efficiency and durability. We are continuing to work to improve these trap designs and have recently developed a 3D printed trap that is like our initial passive trap in concept, but more durable for long-term monitoring (Supp Fig. 1 [online only]). This trap allows us the potential efficiency of the initial passive trap's capture success (0.87 [95% CI = 0.62, 0.96]), while maintaining the durability of the motorized trap and ease of use for downstream DNA processing, and they are easier to deploy and exchange slides than our initial versions (time to change out slides < 5 min). This trap is also low in cost with printing materials averaging $3 per trap, and each trap is <$10 total for all materials (with mounting stakes and hardware). One additional benefit of using 3D printing technology is that they can also be printed in a variety of colors, so they can blend into the landscape if deployed in federal wilderness areas. Certainly, there is potential for future research to design more traps outside of what we have tested or improve upon any of these designs for eDNA-based monitoring. It could also be beneficial to further study other environmental variables that may affect the success of a trap capturing HWA, such as wind direction and hemlock density. Ultimately, fully eDNA-compatible traps that allow for quick processing time could be an efficient method for land managers to detect early infestations and low-density HWA populations that can be difficult to identify visually. As we move into the future of using airborne eDNA for invasive species monitoring, using these methods not only for presence/absence detection, but also to gain quantitative information on abundance or infestation levels (via qPCR or amplicon sequencing) (Kirtane et al. 2022) becomes a real possibility. As these traps continue to be refined, they would be a useful additional tool in the manager's toolbox for early monitoring of HWA.

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Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

Supplemental Fig. 1. Photo of a 3D printed trap design that is more like our initial passive trap in concept with more durability for long-term monitoring currently being used in our continued research of efficient HWA eDNA traps.

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