Journal of Economic Entomology, 2026, **00**(0), 1–12
DOI: https://doi.org/10.1093/jee/toag202
Research

Biological and Microbial Control

Effect of *Laricobius* spp. (Coleoptera: Derodontidae) and *Leucotaraxis piniperda* (Diptera: Chamaemyiidae) predation on hemlock woolly adelgid (Hemiptera: Adelgidae) and on eastern hemlock (Pinales: Pinaceae) health in the eastern United States

Carrie E. Preston*[,1], Timothy Shively[2], Mark C. Whitmore[3], Jerome F. Grant[4], Albert Mayfield III[5], Timothy Tomon[6], Nathan P. Havill[7], Zephyr Zembrzuski[7], John Seiler[8], and Scott M. Salom[1]

[1]Department of Entomology, College of Agriculture and Life Sciences, Virginia Tech, Blacksburg, VA, USA
[2]Department of Plant, Soil, and Microbial Sciences, School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA, USA
[3]Department of Natural Resources and the Environment, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY, USA
[4]Department of Entomology and Plant Pathology, Institute of Agriculture, University of Tennessee, Knoxville, TN, USA
[5]USDA Forest Service, Southern Research Station, Asheville, NC, USA
[6]USDA Forest Service, Morgantown Field Office, Morgantown, WV, USA
[7]USDA Forest Service, Northern Research Station, Hamden, CT, USA
[8]Department of Forest Resources and Environmental Conservation, College of Natural Resources and Environment, Blacksburg, VA, USA

*Corresponding author. Department of Entomology, Virginia Tech, Dansville, NY 14437, USA (Email: carpresto2010@gmail.com).

Subject Editor: Michael Brewer

In the eastern United States, feeding by introduced predators *Laricobius nigrinus* Fender and *Laricobius osakensis* Shiyake and Montgomery are likely insufficient to sustainably regulate the invasive hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, due to adelgid population rebound postpredation. This suggests that additional predators may be needed in eastern North America to achieve effective biological control. In the Pacific Northwest, there is evidence that multiple specialist predators, including *La. nigrinus, Leucotaraxis piniperda* (Malloch), and *Le. argenticollis* (Zetterstedt) contribute to maintaining HWA populations below damaging levels on western hemlock. We report on a 2-yr, 5-location study to evaluate the combined effect of predators, *La. nigrinus, La. osakensis*, and *Le. piniperda* on HWA populations and eastern hemlock health in the eastern United States. Three assessments targeting different stages of predator-prey activity were conducted annually by installing mesh cages on HWA-infested branches where *La. nigrinus* was established. Predation by *Laricobius* spp. caused measurable spring HWA ovisac disturbance, and *Le. piniperda* predation caused measurable summer HWA ovisac disturbance during both years. Proportion of new shoot growth and mean length of new shoot growth did not differ among treatments for both years. This suggests that a longer-term study may be needed to better understand the combined effects these predators may have on eastern hemlock health, or that additional predators such as *Le. argenticollis* should also be evaluated.

Keywords: classical biological control, eastern hemlock, adelgid, invasive species

Introduction

Managing the impact and spread of the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), in eastern North America is a continuing challenge. This insect was first recorded in eastern North America in 1951 in Richmond, VA (Stoetzel 2002), and was likely transported on nursery stock sent from Japan (Havill and Montgomery 2008, Havill et al. 2016). Since the 1980's, HWA has spread to 23 US states and 2 Canadian provinces (Nova Scotia and Ontario) (Dodd 2023). It has been a successful invader due in part to its parthenogenesis, high fecundity, multivoltinism (McClure 1989, Havill et al. 2016), and lack of specialized natural enemies in its introduced range (Wallace and Hain 2000). In eastern North America, HWA feeds on 2 susceptible hosts, the eastern hemlock, *Tsuga canadensis* (L.) Carrière (Pinales: Pinaceae), and Carolina hemlock, *Tsuga caroliniana* Engelm (Pinales: Pinaceae) (McClure 1989).

Received: 1 April 2024. **Revised:** 19 May 2026. **Accepted:** 19 June 2026

Eastern hemlock has a wide distribution in eastern North America, extending from Nova Scotia into Alabama and west into Wisconsin, with isolated populations in Minnesota, southern Michigan, western Ohio, southern Indiana, and Kentucky (Godman and Lancaster 1990). Eastern hemlock grows in pure stands or mixed-species stands and is considered a major component in 4 mixed-stand types (Godman and Lancaster 1990). The distribution of Carolina hemlock is substantially smaller; it overlaps with eastern hemlock, with isolated populations in Georgia, North Carolina, South Carolina, Virginia, and Tennessee (Godman and Lancaster 1990). Eastern hemlock is a foundation species (Ellison 2014), creating unique and stable ecosystems with over 120 vertebrate species and at least 300 arthropod species (Dilling et al. 2009, Ellison et al. 2018). It is also the most long-lived, shade tolerant tree species in eastern North America (Farjon 1990). Without eastern and Carolina hemlocks, these unique ecosystems would be lost, resulting in elevated understory light levels, negative effects on several bird species, alterations in arthropod diversity and abundance, and alterations in nutrient and energy flow (Ellison et al. 2018).

HWA feeding causes plant chlorosis, premature needle drop, bud abortion, reduction of new growth, and branch dieback (McClure et al. 1996). Physiological effects of herbivory on the host are variable depending on the scale of measurement and may include an increase (Huggett et al. 2018) or a decrease (Domec et al. 2013) in hydraulic conductivity, higher net photosynthetic rates (Huggett et al. 2018), or reduced net photosynthetic rates (Domec et al. 2013, Nelson et al. 2014, McDonald et al. 2023), and a reduction in stomatal conductance depending on additional environmental stressors (Preston et al. 2023a). As a result of these physical and physiological effects, tree mortality may occur at any age after 4 or more years of infestation (McClure et al. 1996).

To manage the impact of HWA in forest stands, classical biological control programs have focused on the release and redistribution of 4 specialist predators. Two specialist predators of HWA, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) and *Laricobius osakensis* Montgomery and Shiyake (Coleoptera: Derodontidae), have established populations in the eastern United States (Mausel et al. 2010, Toland et al. 2018, Crandall et al. 2023). In its introduced range, HWA has 2 generations per year: the spring generation known as the sistens, and the summer generation known as the progrediens (McClure 1989). Both *La. nigrinus* and *La. osakensis* target the spring HWA generation (sistens) and feed on its eggs (progrediens) (Vieira et al. 2012). *Laricobius nigrinus* can significantly reduce ovisac density of the sistens generation (Jubb et al. 2020), resulting in a positive temporary effect on tree physiology (Preston et al. 2023a). However, these specialist predators only target the sistens adults and progrediens eggs, and 1 study by Crandall et al. (2020) determined that even though there were high predation rates of sistens ovisacs (up to >80%) by *La. nigrinus*, the low number of surviving progrediens would still be enough to cause damage to the tree when the mother sistens generation density was high. Predators that also target sistens eggs, *Leucotaraxis argenticollis* (Zetterstedt) (Diptera: Chamaemyiidae) and *Leucotaraxis piniperda* (Malloch) (Diptera: Chamaemyiidae), also known as silver flies, have recently been released in the eastern United States (Limbu et al. 2018). Both of these species co-occur with *La. nigrinus* in the US Pacific Northwest and are known to feed on both generations of HWA (Kohler et al. 2008, Grubin et al. 2011, Kohler et al. 2016, Rose et al. 2020, Dietschler et al. 2021). Eastern strains of both species are found in the eastern United States, but these strains are known to feed on other adelgid species besides HWA and are phylogenetically divergent from the western strains (Havill et al. 2018, 2023). Efforts to monitor for establishment of the western strains of both *Leucotaraxis* species in the eastern United States are ongoing.

In western North America, *Le. argenticollis* emerges prior to *La. nigrinus* prepupal drop (Dietschler et al. 2021). *Leucotaraxis piniperda* emerges at the end of HWA sistens oviposition and after *La. nigrinus* prepupal drop (Dietschler et al. 2021). There is also a second, lesser emergence period for *Le. argenticollis*, following *Le. piniperda* emergence (Dietschler et al. 2021). With emergence occurring at separate times for each species, it is possible to differentiate the 2 *Leucotaraxis* species based on this timing. In addition, *Le. argenticollis* and *Le. piniperda* can tolerate environmental conditions in New York and Tennessee during the spring through early summer (Motley et al. 2017), and *Le. argenticollis* is capable of overwintering as puparia in plant hardiness zones 4a to 7a (Dietschler et al. 2023).

Since *Le. piniperda* emerges after *La. nigrinus* prepupal drop and near the end of HWA sistens oviposition, it is likely that *Le. piniperda* mainly targets the HWA progrediens generation. By releasing *Le. piniperda* at field sites where *La. nigrinus* has established, it is possible that the presence of both predator species could have a greater effect on HWA populations than either one in isolation. After *La. nigrinus* prepupal drop occurs, *Le. piniperda* emergence occurs making it possible to determine the effects of *La. nigrinus* on the sistens generation separately from the effects of *Le. piniperda* on the progrediens generation. For this study, *Le. piniperda* was experimentally added to caged release treatments at sites with established *La. nigrinus* populations. This study was composed of 3 time-specific assessments with the following objectives: Assessment 1, to determine sistens winter mortality and the effect of *La. nigrinus* on sistens ovisacs; Assessment 2, to determine progrediens summer mortality and the impact of *Le. piniperda* on progrediens ovisacs; and Assessment 3, to determine the overall impact of both predators, separately and in combination, on the proportion of new shoot growth, the mean length of new shoot growth, and the density of aestivating sistens on new shoot growth.

Materials and Methods

Field Site Set Up

This 2-yr study was conducted at 5 field sites, each in a different eastern US state (Table 1). Each site was selected based on having an active infestation of HWA, releases of *La. nigrinus* beginning in 2005 to 2007, and documented establishment of *La. nigrinus* for at least 4 yrs prior to the study. *Laricobius osakensis* was also released at the TN site, but was not known to have established before our study began. In the fall of each year (2020 and 2021), when HWA sistens nymphs resumed development following summer aestivation, 8 to 10 trees were selected at each site. Trees selected were of similar crown classification (intermediate and/or suppressed) (Table 1) (Schomaker et al. 2007).

On each tree, 8 branches were selected based on HWA sistens density on current year shoot growth. Four branches with >1 HWA/cm were randomly assigned to one of the 4 following

Table 1. Field site information for eastern hemlock stands used to evaluate predator impact on hemlock woolly adelgid in the eastern United States, 2021 to 2022

Site	Location coordinates	Plant hardiness zone[a]	Release year of *Laricobius nigrinus*	Release year of *Laricobius osakensis*[b]	Tree crown classification
MD	39.70 N, −78.67 W	7a	2004	N/A	Suppressed, intermediate
NC	35.82 N, −82.19 W	7a	2005	N/A	Suppressed
PA	40.75 N, −77.72 W	6b	2006	N/A	Intermediate
TN	35.76 N, −83.30 W	7b	2007	2014	Suppressed
VA	37.64 N, −78.80 W	7b	2005	N/A	Suppressed, intermediate

[a]Plant hardiness zone assignments based on https://planthardiness.ars.usda.gov/.
[b]N/A indicates that *La. osakensis* was not released at the site.

Table 2. Treatment codes and characteristics for branches on eastern hemlock trees used to evaluate predators and cage effects on hemlock woolly adelgid and eastern hemlock health in the eastern United States, 2021 to 2022

Treatment[a]	HWA density	Apply cage[b]	Remove cage[c]
Both predators	>1 HWA/cm	Assessment 1	End of study
No predators	>1 HWA/cm	Start of study	Assessment 1
Ln only	>1 HWA/cm	No cage	No cage
Lp only	>1 HWA/cm	Start of study	End of study
Spring cage	<1 HWA/cm	Assessment 1	End of study
Fall cage	<1 HWA/cm	Start of study	Assessment 1
No cage	<1 HWA/cm	No cage	No cage
Fall and spring cage	<1 HWA/cm	Start of study	End of study

[a]"Ln only" represents the treatment with *La. nigrinus*. "Lp only" represents the treatment with *Le. piniperda*.
[b]Cages were applied when the study began for the year, or after the first branch was clipped for Assessment 1.
[c]Cages were removed after the first branch was clipped for Assessment 1, or when the study was completed for the year.

predator treatments, defined by adelgid exposure to predation by *La. nigrinus* (Ln) and/or *Le. piniperda* (Lp): (i) No predators; (ii) Lp only; (iii) Both predators; and (iv) Ln only (Table 2). Three 20 to 30 cm branchlets with similar HWA densities (>1 HWA/cm) were marked on each of the 4 branches per tree. Each branchlet was randomly assigned to one of the 3 time-specific assessments of the study (described below). In treatments with *La. nigrinus* predation (Ln only and Both predators), branches remained exposed to naturally occurring *La. nigrinus* populations from fall through early spring, whereas treatments without *La. nigrinus* predation, a 100 × 66 cm (L × W) nylon mesh cage (MegaView Science Co., Ltd., Taichung, Taiwan) was installed around the branch to prevent *La. nigrinus* access. For treatments with *Le. piniperda* predation (Lp only and Both predators), adult *Le. piniperda* were released into a mesh cage surrounding the branch in the spring, whereas for treatments without *Le. piniperda*, they were not. Four branches with <1 HWA/cm (to represent negligible adelgid impact on shoot growth), each with three 20 to 30 cm branchlets with similar HWA densities, were also selected as cage treatments with cages installed at the same times as their corresponding predator treatment branches (Table 2). Before mesh cages were applied, branches that were assigned to the predator treatments and corresponding cage treatments without *La. nigrinus* (Lp only and No predators) were tapped approximately ten times to remove any natural-occurring predators of HWA that might have been present. The neck of each cage was closed over a piece of insulated pipe foam 7.5 cm long by 1.25 cm wide and secured with zip ties.

Experiment Set up and *Le. piniperda* Adult Releases

At the New York State Hemlock Initiative (NYSHI) facility, at Cornell University, *Leucotaraxis* that emerged from foliage material shipped from sites in the Pacific Northwest, were collected and identified to species by observing whether postpronotal setae were present (*Le. argenticollis*) or absent (*Le. piniperda*) (Dietschler et al. 2021, Gaimari and Havill 2021). Adult silver flies identified as *Le. piniperda* were then sexed by observing the genitalia under a dissecting scope (Dietschler et al. 2021). Adult silver flies were then sorted and shipped overnight to collaborators in MD, PA, NC, TN, and VA. The MD and VA sites were monitored by the senior author.

The *La. nigrinus* sampling periods map from the HWA Predator Database (Dodd 2023) was used to predict the timeframe of *La. nigrinus* prepupal drop at each site for each year. This map was based on a phenological model of *La. nigrinus* that included data on the survival and development of this species (Rémi Saint-Amant, personal communication, 7 April 2026). Within the timeframe, 1 branchlet was clipped for all predator and cage treatments and returned to a lab in each respective state. Cages were removed for the No predator treatment and corresponding cage treatment branches. On the Both predators and Lp only treatment branches, *Le. piniperda* adults were released and cages were applied. Cages were also applied to corresponding cage treatment branches. *Leucotaraxis piniperda* adults were released in M: F sex ratios of 5:5 and 4:4 in 2021 and 2022, respectively. The NC site was monitored by author A.M., the PA site was monitored by author T.T., and the TN site was monitored by author J.G. Cages remained on Lp only treatment and corresponding cage treatment branches for the remainder of the study.

Assessment 1: *La. nigrinus* Impact and HWA Winter Mortality

Assessment 1 determined the effect of *La. nigrinus* on HWA sistens ovisacs, assessed HWA sistens natural winter mortality, and quantified *Laricobius* spp. larvae present. At the respective labs, cut branchlets were held in small containers that mimicked modified Burlese funnels to collect *La. nigrinus* after they had reached the prepupal stage (Salom et al. 2012, Foley et al. 2021). The containers consisted of 0.11 kg mason jars with wire mesh lining the inside of the lid, which allowed *La.*

nigrinus prepupae to fall through to the bottom of the jars. The cut ends of the branches were placed in water-saturated floral foam (Smithers-Oasis North America, Kent, OH). Thin clear plastic surrounded each branch and stood on top of the wire mesh. All containers were held in an environmental chamber at 13 °C with a 12 L: 12 D photoperiod. Containers were checked daily to collect *La. nigrinus* prepupae, which were then stored in 95% EtOH and at −20 °C. When *La. nigrinus* prepupae stopped dropping for 5 d, branches were placed at −20 °C until evaluation for impact on HWA. Branchlets collected from MD, PA, and VA sites were examined at Virginia Tech, whereas branchlets collected from NC and TN sites were examined in the labs of co-authors A.M. and J.G., respectfully.

All predator and cage treatment branches were analyzed to determine the impact of *La. nigrinus* on HWA sistens ovisacs and to assess HWA sistens winter mortality. From 1-yr old shoot growth on each branchlet, the number of disturbed HWA sistens ovisacs, live HWA sistens, and dead HWA sistens were recorded. HWA sistens were considered live if their woolly ovisacs were intact and if they produced red hemolymph when prodded. HWA sistens were considered dead if their woolly ovisacs were intact but the insect was shrunken and dry or produced dark brown hemolymph when prodded. HWA sistens ovisacs were considered disturbed from *La. nigrinus* predation if the ovisac had a shredded appearance (Jubb et al. 2020). Ovisac disturbance has been used in multiple studies as an indicator of *La. nigrinus* predation (Mausel et al. 2008, Jubb et al. 2020), as it is the only known predator active at this time of year (Mayfield et al. 2023). Percent live HWA sistens was determined by dividing the number of live HWA by the total number of HWA sistens counted at the time of the assessment. Percent winter mortality was determined by dividing the number of dead (with intact ovisacs) HWA sistens by the total number of HWA sistens counted, and the percent disturbed HWA sistens was determined by dividing the number of disturbed HWA sistens by the total number of HWA sistens counted at the time of the assessment.

Assessment 2: *Le. piniperda* Impact and HWA Summer Mortality

Assessment 2 evaluated HWA progrediens summer mortality and the effect of *Le. piniperda* on HWA progrediens ovisacs. In June or July (depending on site location), when the majority of progrediens adults had produced most of their eggs, the second of the 3 experimental branchlets was cut from each predator treatment and corresponding cage treatment branch and transported to the lab. Branchlets were held in the freezer at −20 °C until they could be evaluated for impact on HWA. For evaluation, progrediens were classified as live, dead, or disturbed using the same methods as in Assessment 1. HWA progrediens nymphs and adults that failed to produce eggs and were dead due to other causes, besides *Le. piniperda* predation, were considered dead due to summer conditions. All *Leucotaraxis* spp. specimens that were found were collected and stored in 95% EtOH and at −20 °C for molecular identification.

Assessment 3: Tree Health Measurements, Settled Sistens Density, and Predator Impacts

Assessment 3 examined the effect of *Laricobius* spp. and *Le. piniperda* predation and the density of the next generation of settled aestivating sistens on new shoot growth. In September and October, while settled sistens were still in aestivation, the final experimental branchlet was cut from all predator treatment and corresponding cage treatment branches. Debris that had gathered in the cages was also collected and examined for *Le. piniperda* specimens. Cages were removed and returned to the lab. Branchlets and debris were frozen at −20 °C until evaluation. *Leucotaraxis piniperda* specimens that were found on the branchlet and in cage debris were collected and stored in 95% EtOH and held in the freezer at −20 °C for molecular identification.

For each branchlet the number of new shoots and the total number of shoots were recorded, and the proportion of new shoot growth (new shoots/total shoots) was calculated. New shoot growth was measured to the nearest 0.1 cm, and mean length of new shoot growth was calculated by dividing the sum of new shoot growth by the number of new shoots. The density of aestivating sistens nymphs was determined by the total number of aestivating sistens on new shoot growth divided by the sum of new shoot growth.

Molecular and Morphological Analyses of Predators

For *Laricobius* and *Leucotaraxis* specimens, DNA was nondestructively extracted using the MagMAX DNA Multi-Sample Kit (Thermo Fisher, Waltham, MA) on a KingFisher Flex instrument (Thermo Fisher). Each individual was pierced with a clean flame-sterilized insect pin, and the body was removed after overnight digestion in tissue lysis buffer. Vouchers of each species were deposited in the Virginia Tech Insect Collection, Blacksburg, Virginia (VTEC) with accession numbers VTEC000010523 to VTEC000010545. The standard DNA barcoding region of the 5′ end of the mitochondrial COI gene was amplified in 25 µL reactions containing 1X PCR Buffer, 2.4 µL dNTPs (10 mM each; New England Biolabs, Ipswich, MA), 3.7 µL MgCl2 (25 mM), 1.0 µL of each primer LepF1 and LepR1 (10 mM; Hebert et al. 2004), 0.2 µL Go Taq G2 DNA polymerase Kit (Promega), and 3 or 5 µL template DNA. The thermocycler profile consisted of 1 cycle at 95 °C for 2 min, then 36 cycles at 95 °C for 30 s, 46 °C for 30 s, and 72 °C for 1 min (30 cycles), and a final extension of 72 °C for 2 min. PCR products were sequenced at the Yale University Keck DNA Sequencing Facility on an ABI 3730 sequencer (Thermo Fisher). Chromatograms were examined and edited using the software GENEIOUS. For identification of immature *Leucotaraxis*, DNA barcode sequences were compared to sequences from adults determined using morphology. All new sequences generated in this study were deposited in GenBank with voucher numbers PP352701 to PP352912. The presence or absence of sclerotization on the puparial posterior spiracles was used to distinguish puparia of *Le. piniperda* from *Le. argenticollis* (Gaimari and Havill 2021) for specimens collected at the NC and PA sites in 2021, and MD site in 2022.

Statistical Analysis

Assessment 1: *La. nigrinus* Impact and HWA Winter Mortality

This study was set up as a randomized complete block design. Site and tree nested within site were set as random variables to account for the variability among the sites and to avoid pseudo replication from pooling the treatments on the same tree. Treatment and year were set as fixed. Response variables consisted of proportions with zeros and ones within the dataset.

Therefore, a generalized linear mixed model with an ordered Beta distribution and logit link was used to analyze the effect of predator treatments on the proportion of disturbed sistens ovisacs, and to analyze the proportion of sistens winter mortality among the predator treatments for 2021 and 2022 (Kubinec 2023, Korhonen et al. 2024). Treatments Both predators and Ln only were pooled into a single treatment, because both treatments were inclusive of *La. nigrinus*, the only specialist predator of HWA sistens adults. Similarly, treatments No predators and Lp only that excluded *La. nigrinus* were pooled into a single treatment for these analyses. Branchlet replicates of treatments No predators and Lp only branches that were observed to have been contaminated with *La. nigrinus* were removed from analyses for all assessments.

To determine the mean proportion of sistens ovisac disturbance from *La. nigrinus* predation, the mean proportion of sistens ovisac disturbance of the No Ln treatment was subtracted from the mean proportion of sistens ovisac disturbance of the Ln treatment. This was to distinguish ovisac disturbance due to *La. nigrinus* predation from the mechanical disturbances that could have occurred to some of the sistens ovisacs on all treatment branches. Possible causes of mechanical disturbance include environmental conditions such as the wind, cages rubbing against the branches, and the handling of the branches while at the site, or by transporting them to the lab. All analyses (Assessments 1 to 3) were conducted in R (R Core Team 2025) using gamlss (Rigby and Stasinopoulos 2005), glmmTMB (Brooks et al. 2017), and supporting packages dplyr (Wickham et al. 2023) and emmeans (Lenth and Piaskowski 2026). Models for all analyses (Assessments 1 to 3) were validated using the DHARMa package (Hartig 2022). Cage treatments were excluded from all Assessments 1 and 2 analyses because they were selected specifically for cage effects on eastern hemlock health. All figures were created using the package ggplot2 (Wickham 2016).

Assessment 2: *Le. piniperda* Impact and HWA Summer Mortality

When the first branchlet from all treatments was clipped for Assessment 1, *La. nigrinus* present on predator treatment branches were assumed to have reached the final larval stage or were prepupae, and no longer feeding on HWA progrediens eggs and HWA sistens. Therefore, for Assessment 2, data were pooled into treatments with *Le. piniperda* (Both predators and Lp only) and without *Le. piniperda* (No predators and Ln only) to determine effects on the proportion of progrediens ovisac disturbance and progrediens summer mortality among the predator treatments. Zeros and ones were present within the dataset, therefore a generalized linear mixed model with an ordered Beta distribution with a logit link was used to analyze predator treatment effects on progrediens ovisac disturbance and progrediens summer mortality to determine differences among treatments across sites for 2021 and 2022. Fixed and random variables in this model were the same as in the model used for Assessment 1, see above. To determine the mean proportion of progrediens ovisac disturbance from *Le. piniperda* predation, the mean proportion of progrediens ovisac disturbance of the No Lp treatment was subtracted from the mean proportion of progrediens ovisac disturbance of the Lp treatment. This was to distinguish ovisac disturbance due to *Le. piniperda* predation from the mechanical disturbances that could have occurred to some of the progrediens ovisacs on all treatment branches. The TN site was excluded from this analysis for both years because branch sampling occurred in July, which was too late in the season to accurately capture predation effects (sistens eggs had already hatched and sistens crawlers dispersed).

Assessment 3: Tree Health Measurements, Settled Sistens Density, and Predator Impacts

The TN site was excluded from the cage treatment analyses for both years, due to initial HWA densities >1 HWA/cm.

A generalized linear mixed model with an ordered Beta distribution with a logit link was used to analyze the effect of predator treatments and cage treatments on the proportion of new shoots in 2021 and 2022. To analyze the effect of predator treatments on the mean length of new shoot growth in 2021 and 2022, a generalized linear mixed model with a zero-inflated gamma distribution with a log link was used. This was to account for the high number of zeros within the dataset for data that was not proportion data. To analyze the effect of cage treatments on the mean length of new shoot growth a linear mixed model was used since the data was not zero inflated or in proportions. For both models, fixed variables were the same as what was used for previous assessments, and site was a random variable. Treatments were not pooled therefore; tree nested within site was not a random variable for this assessment.

Due to a low number of new shoots that were produced across treatments and sites, we were not able to analyze HWA density on new shoot growth.

Results

Assessment 1: *La. nigrinus* Impact and HWA Winter Mortality

In 2021, mean initial HWA densities for all predator treatments ranged between 1.80 and 4.38 HWA/cm across all sites (Supplementary Table S1). In 2022, mean initial HWA densities ranged between 2.82 and 5.05 HWA/cm across all sites (Supplementary Table S1).

Overall, sistens ovisac disturbance in the Ln treatment was significantly greater than in the No Ln treatment ($z = -9.75$, $P < 0.0001$, Fig. 1). Overall, sistens ovisac disturbance in 2022 was significantly greater compared to 2021 ($z = 6.28$, $P < 0.0001$, Fig. 1). In 2021, sistens ovisac disturbance, from *Laricobius* spp. predation, ranged between 11.8% and 53.2% among all sites (Supplementary Table S2). In 2022, the mean proportion of sistens ovisac disturbance from *Laricobius* spp. predation, ranged between 13.0% and 49.7% among all sites (Supplementary Table S2).

There was modest evidence that sistens winter mortality was greater in the No Ln treatment compared to the Ln treatment ($z = -3.39$, $P = 0.03$, Fig. 2). Overall, sistens winter mortality was significantly lower in 2022 than in 2021 ($z = -3.39$, $P = 0.0007$, Fig. 2). In 2021, mean sistens winter mortality was <50% in MD and PA, as low as 13.4% in NC, and as high as 73.9% in TN. In 2022, mean sistens winter mortality was <50% for all field sites and was as low as 0.9% in TN.

The molecular analyses determined that *La. nigrinus* was present at all field sites (Table 3). However, at the TN site, 72% ($n = 18$) were *La. osakensis*, 17% were *La. nigrinus*, and 11% were *La. rubidus* LeConte (Coleoptera: Derodontidae) (Table 3). The greatest number of *Laricobius* larvae that were

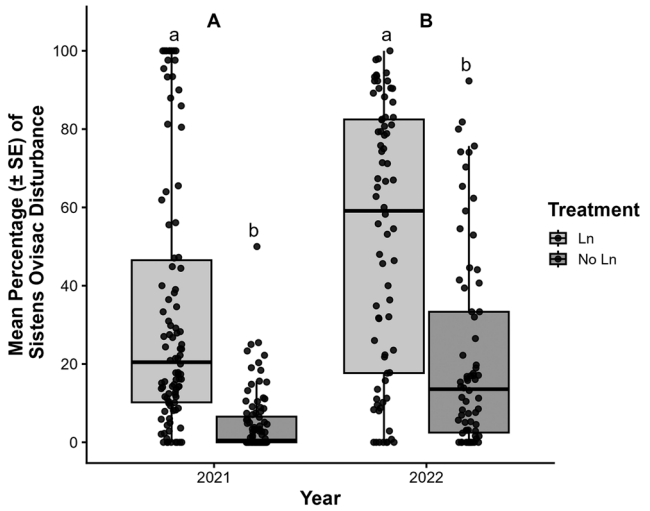

Fig. 1. Assessment 1: Ovisac disturbance of the sistens generation of *Adelges tsugae*. Mean percentage (±SE) of disturbed sistens on predator treatment branches. Different lowercase letters above individual box plots indicate significant differences between treatments for each year ($P < 0.05$). Different capitalized letters above box plots indicate significant differences between years ($P < 0.05$). Data points represent data points across all sites for each treatment. "Ln" represents treatment branches exposed to *La. nigrinus* and "No Ln" represents treatment branches without *La. nigrinus*.

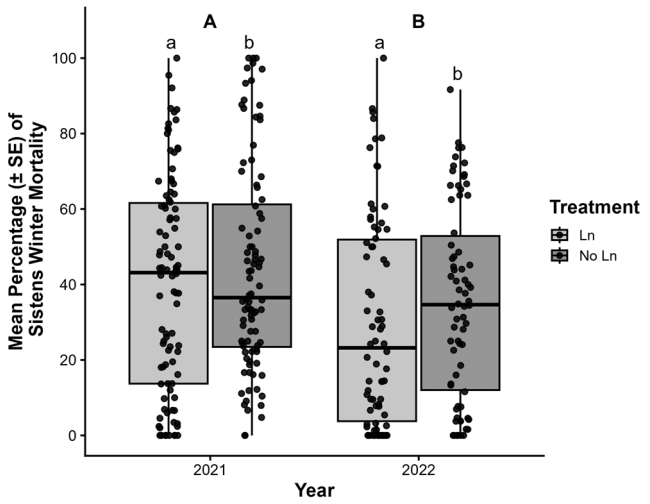

Fig. 2. Assessment 1: Winter mortality of the sistens generation of *Adelges tsugae*. Mean percentage (±SE) of sistens winter mortality on predator treatment branches. Different lowercase letters above individual box plots indicates significant differences among treatments ($P < 0.05$). Different capitalized letters above box plots indicate significant differences between years ($P < 0.05$). Data points represent data points across all sites for each treatment. "Ln" represents treatments with *La. nigrinus* and "No Ln" represents treatments without *La. nigrinus*.

collected occurred in 2021 at the NC site with a total of 105 *Laricobius* larvae (Table 4). The lowest number of *Laricobius* larvae that were collected occurred in 2021 at the PA site with a total of 2 *Laricobius* larvae collected (Table 4).

Assessment 2: *Le. piniperda* Impact and HWA Summer Mortality

In 2021, mean initial HWA densities for all predator treatments ranged between 1.93 and 4.30 HWA/cm across all sites (Supplementary Table S1). In 2022, mean initial HWA densities ranged between 2.84 and 6.57 HWA/cm across all sites (Supplementary Table S1).

Overall, progrediens ovisac disturbance in the No Lp treatment was significantly lower compared to the Lp treatment ($z = -2.94$, $P = 0.003$, Fig. 3). Overall, progrediens ovisac disturbance in 2022 was significantly greater than in 2021 ($z = 4.62$, $P < 0.0001$, Fig. 3). In 2021, the mean percentage of progrediens ovisac disturbance from *Le. piniperda* predation, ranged from 2.9% to 8.3% across all sites (Supplementary Table S3). In 2022, progrediens ovisac disturbance from *Le. piniperda* predation, ranged from 0.0% to 13.3% across all sites (Supplementary Table S3).

Overall, progrediens summer mortality in the No Lp treatment was not significantly different from the Lp treatment ($z = -1.07$, $P = 0.29$, Fig. 4). However, overall progrediens summer mortality was significantly greater in 2021 than in 2022 ($z = -5.89$, $P < 0.0001$, Fig. 4). In 2021, mean progrediens summer mortality ranged from 9.6% in NC to 78.8% in PA. In 2022, mean progrediens summer mortality ranged from 11.0% in VA to 43.4% in NC.

Molecular analyses confirmed that the western strain of *Le. piniperda* was collected from cages from all field sites, except NC (Table 3). Specimens collected from the NC site were mostly puparia from which adults had emerged, so they did not contain enough DNA to be analyzed molecularly. These specimens were confirmed to be *Le. piniperda* using morphological identification, yet it was not possible to determine if they were the western strain. Molecular analyses confirmed that all specimens except one, from VA in 2022, were the western strain of *Le. piniperda* (Table 3). In addition, adult flies were released into mesh cages and confined within those cages, preventing the eastern strain of *Le. piniperda* from being actively present on those branches. Therefore, we can assume that all specimens confirmed to species based on morphological characteristics were also the western strain. In 2022, at the VA field site, 97.1% of *Leucotaraxis* specimens collected were the western strain of *Le. piniperda* ($n = 34$) and 2.9% were the western strain of *Le. argenticollis* ($n = 1$) (Table 3). The majority of specimens collected from all sites were undetermined due to low presence of DNA or contamination (Table 3). Morphological analyses concluded that all emerged puparia were *Le. piniperda* (Table 3).

Assessment 3: Tree Health Measurements and Predator Impacts

In 2021, mean initial HWA densities for all predator treatment branches ranged between 2.15 and 3.61 HWA/cm across all sites (Supplementary Table S1). Mean initial HWA densities for cage treatment branches ranged between 0.13 and 0.41 HWA/cm across all sites (Supplementary Table S4). In 2022, mean initial HWA densities for predator treatment branches ranged between 2.98 and 5.82 HWA/cm across all sites (Supplementary Table S1). Mean initial HWA densities for cage treatment branches ranged between 0.16 and 0.77 HWA/cm across all sites (Supplementary Table S4).

The proportion of new shoot growth was not significantly different among predator treatments (Ln only: $z = -1.20$, $P = 0.23$, Lp only: $z = -1.63$, $P = 0.10$, and No predators: $z = -1.29$, $P = 0.20$) (Fig. 5). Overall, the proportion of new shoot growth was significantly greater in 2022 compared with 2021 ($z = 3.77$, $P = 0.0002$). In 2021, the mean percentage of new shoot growth ranged between 1.2% and 42.4% among all sites and predator treatments. In 2022, the mean

Table 3. Molecular and morphological results of *Laricobius* spp. and *Leucotaraxis* spp. specimens collected at each site from treatment branches and cage debris, 2021 to 2022

Year	State	Total specimens tested (molecular)[a]	Total specimens tested (morphology)[b]	Number of specimens by type						
				Undetermined[c]	Ln[d]	Lr[e]	Lo[f]	La[g]	Lp[h]	Other[i]
2021	TN	9	0	2	.	.	.	0	2	*5*
2022	TN	43	0	14	3	2	13	0	1	10
2021	NC	29	9	8(8)[j]	21(21)[j]	0	0	0	9(0)[j]	0
2022	NC	0	0							
2021	PA	9	2	6(6)[j]	0	0	0	0	4(2)[j]	1(1)[j]
2022	PA	59	0	46	3	0	0	0	8	2
2021	VA	22	0	9	0	0	0	0	13	0
2022	VA	103	0	23	42	0	0	1	34	3
2021	MD	29	0	19	2	0	0	0	3	*5*
2022	MD	35	11	3(3)[j]	32(32)[j]	0	0	0	11(0)[j]	0

[a]Total specimens tested (molecular) represents the number of specimens that were analyzed using PCR techniques.
[b]Total specimens tested (morphology) represents the number of specimens that were analyzed based on morphological characteristics.
[c]Undetermined represents the number of specimens that could not be identified due to contamination or low amounts of DNA present.
[d]Ln represents the number of *La. nigrinus* specimens that were confirmed to species.
[e]Lr represents the number of *La. rubidus* that were confirmed to species.
[f]Lo represents the number of *La. osakensis* that were confirmed to species.
[g]La represents the number of *Le. argenticollis* that were confirmed to species.
[h]Lp represents the number of *Le. piniperda* that were confirmed to species.
[i]Other represents the total number of specimens that were confirmed to family and were not Ln, Lr, Lo, La, or Lp.
[j]Total number of specimens tested (number of specimens tested molecularly).

Table 4. Assessment 1: Total number of *Laricobius* spp. larvae collected from Ln only and Both predators treatment branches at each site for year 2021 and 2022

Year	Site	Total number of *Laricobius* spp. larvae collected
2021	MD	48
2021	NC	105
2021	PA	2
2021	TN	33
2021	VA	16
2022	MD	22
2022	NC	37
2022	PA	6
2022	TN	20
2022	VA	31

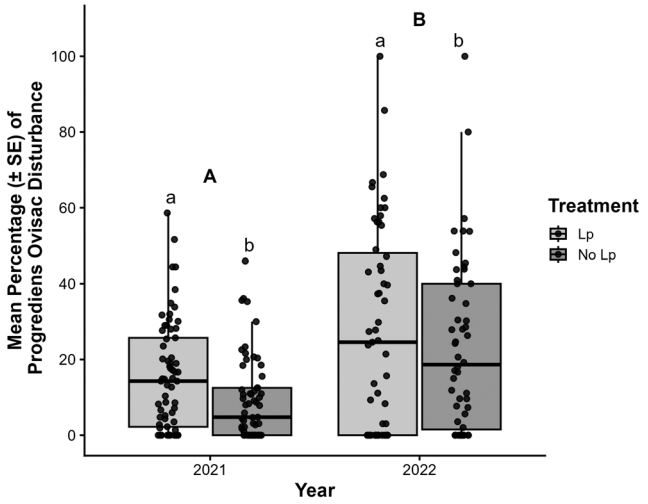

Fig. 3. Assessment 2: Ovisac disturbance of the progrediens generation of *Adelges tsugae*. Mean percentage (±SE) of progrediens disturbed on predator treatment branches. Different lowercase letters above individual box plots indicate significant differences between treatments ($P < 0.05$). Different capitalized letters above box plots indicate significant differences between years ($P < 0.05$). Data points represent data points across all sites for each treatment. Data from treatments with *Le. piniperda* were pooled and assigned "Lp." Data from treatments without *Le. piniperda* were pooled and assigned "No Lp."

percentage of new shoot growth ranged between 7.3% and 58.4% among all sites and predator treatments. The proportion of new shoot growth was not significantly different among the cage treatments (Fall cage: $z = 0.56$, $P = 0.59$, No cage: $z = 0.71$, $P = 0.49$, and Spring cage: $z = 0.28$, $P = 0.78$, Fig. 6). Overall, the proportion of new shoot growth was significantly greater in 2022 compared with 2021 ($z = 3.62$, $P = 0.0003$). In 2021, the mean percentage of new shoot growth ranged between 0.76% and 78.1% across all sites and cage treatments. In 2022, the mean percentage of new shoot growth ranged between 27.1% and 77.6% across all sites and cage treatments.

The mean length of new shoot growth did not significantly differ among predator treatments (Ln only: $z = 0.47$, $P = 0.64$, Lp only: $z = -0.73$, $P = 0.47$, and No predators: $z = 0.28$, $P = 0.78$, Fig. 6). Overall, the mean length of new shoot growth was not significantly different between years ($z = 0.18$, $P = 0.86$). In 2021, the mean length of new shoot growth ranged between 0.25 and 2.57 cm across all sites and predator treatments. In 2022, the mean length of new shoot growth ranged between 0.62 and 3.09 cm across all sites and predator treatments.

The mean length of new shoot growth did not significantly differ among cage treatments (Fall cage: $z = 1.60$, $P = 0.11$, No cage: $z = 0.86$, $P = 0.39$, and Spring cage: $z = 1.62$, $P = 0.10$). Overall, the mean length of new shoot growth was significantly greater in 2022 than in 2021 ($z = 2.76$, $P = 0.006$). In 2021, the mean length of new shoot growth ranged between 0.43 and 3.07 cm across all sites and cage treatments. In 2022, the mean

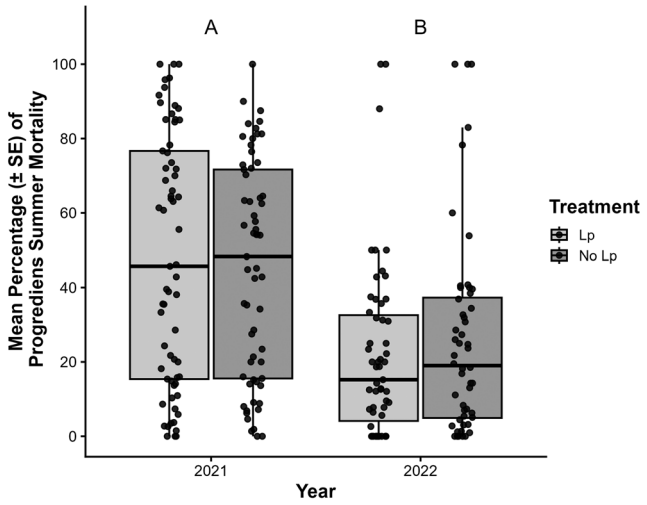

Fig. 4. Assessment 2: Summer mortality of the progrediens generation of *Adelges tsugae*. Mean percentage (±SE) of dead progrediens due to summer conditions on predator treatment branches. No lowercase letters above individual box plots indicates no significant differences among treatments ($P > 0.05$). Different capitalized letters above box plots indicates significant differences between years ($P < 0.05$). Data points represent data points across all sites for each treatment. Data from treatments with *Le. piniperda* were pooled and assigned "Lp." Data from treatments without *Le. piniperda* were pooled and assigned "No Lp."

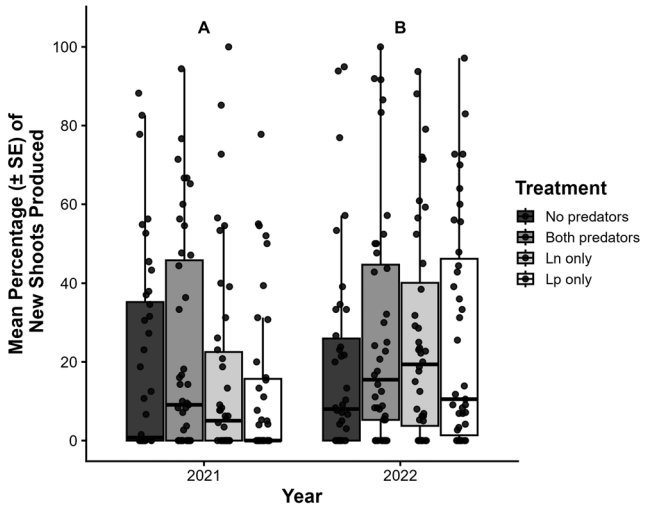

Fig. 5. Assessment 3: Mean percentage (±SE) of new shoot growth (predator treatments). No lowercase letters above individual box plots indicates no significant differences among treatments ($P > 0.05$). Different capitalized letters above box plots indicates significant differences between years ($P < 0.05$). Data points represent data points across all sites for each treatment.

length of new shoot growth ranged between 1.17 and 3.14 cm across all sites and cage treatments.

Discussion

Laricobius spp. predation had a significant effect on sistens ovisac disturbance, and the predation of *Le. piniperda* had a modest effect on progrediens ovisac disturbance. However, our experiments did not demonstrate an effect of *Laricobius* spp. or *Le. piniperda* predation (alone or in combination) on hemlock shoot growth, which we used as an indicator of tree health.

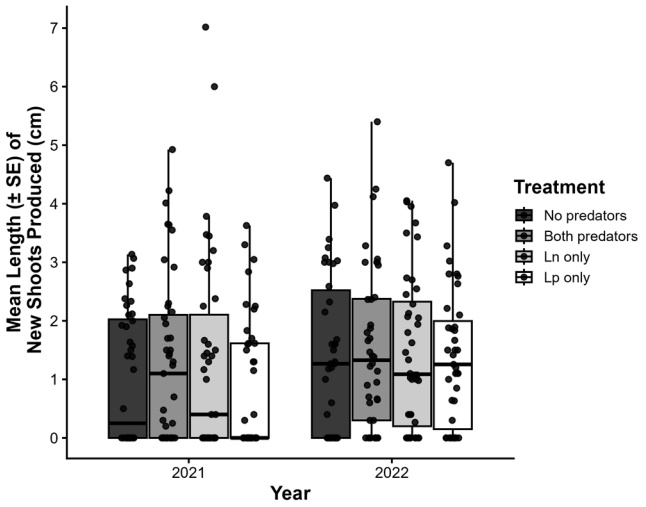

Fig. 6. Assessment 3: Mean length (±SE) of new shoot growth (cm) (predator treatments). No lowercase letters above individual box plots indicates no significant differences among treatments ($P > 0.05$). No capitalized letters above box plots indicates no significant differences between years ($P > 0.05$). Data points represent data points across all sites for each treatment.

Since predation on HWA did not lead to an effect on eastern hemlock health at the branch level, it could indicate that a larger-scale or longer-term study is needed to improve our understanding of these predators and how they affect HWA populations and eastern hemlock health in the eastern United States. It also suggests evaluating additional predators, such as *Le. argenticollis*, which could provide increased pressure on high HWA densities. This study provides a preview into the effects that *Laricobius* and *Leucotaraxis* could have on HWA populations and suggests that continued efforts on the evaluation of their impact on HWA populations and eastern hemlock health in the eastern United States are warranted.

Laricobius spp. and *Le. piniperda* Effects to HWA Populations

Laricobius spp. predation significantly affected sistens ovisacs for both years. These results were consistent with the findings of Jubb et al. (2020) and Preston et al. (2023a), who demonstrated that, established populations of *La. nigrinus* could reduce the HWA sistens population. At the TN field site, the majority (72.7%) of *Laricobius* larvae that were found on treatment branches at the site were confirmed as *La. osakensis* (Table 3). Although, predation by established populations of *La. osakensis* predation on sistens ovisacs has not been previously quantified, our results at the TN field site suggest that *La. osakensis* predation causes similar disturbance of HWA sistens ovisacs as *La. nigrinus*.

Progrediens ovisac disturbance was significantly greater for treatments with *Le. piniperda* compared to treatments without *Le. piniperda* for both years (Fig. 3). Progrediens ovisac disturbance caused by *Le. piniperda* was low ranging from 2.9% to 8.3% in 2021, and 0% to 13.3% in 2022 across all sites. This was lower compared to sistens ovisac disturbance caused by *Laricobius* spp., which ranged between 11.9% and 53.2% in 2021 and 13.0% and 49.7% in 2022 across all sites. For this study, an unknown number of wild *La. nigrinus*, was able

to approach treatment branches. *Leucotaraxis piniperda* was not established at any of our field sites, so we had to select a specific number of adult flies to release into mesh cages. The optimal number of adult *Le. piniperda* that should be released to effectively reduce the progrediens population is uncertain. Another possibility for why progrediens ovisac disturbance was considerably lower in the Lp treatments would be the time of release of adult *Le. piniperda* into cages. In the US Pacific Northwest, Dietschler et al. (2021) found that *Le. piniperda* adult emergence occurred after *La. nigrinus* prepupal drop. For this study, *Le. piniperda* adult flies were released into cages while *La. nigrinus* prepupal drop was still occurring. This was done to limit the number of times we opened and shut the cages to minimize any potential damage to the study branches and from mechanically disturbing HWA. Therefore, when *La. nigrinus* prepupal drop was occurring, the branchlet for Assessment 1 could be clipped at the same time when *Le. piniperda* adult flies were released into cages. As a result, it is possible *Le. piniperda* adult flies may have been released into the cages too soon and their phenology may not have been synchronized with HWA phenology at a field site. It is important to note that while HWA ovisac disturbance has been used as a proxy for HWA population impact it is only a rough estimate of actual population impact because the number of eggs consumed per ovisac is variable (Jubb et al. unpublished data). When HWA densities are high (>1 HWA/cm) it can also be difficult to distinguish the exact number of ovisacs that were disturbed, so an estimate on the number of ovisacs disturbed is produced. Trained and experienced researchers collected these data to remain consistent and to reduce bias.

One adult silver fly collected from treatment branches at the VA site was determined as the western strain of *La. argenticollis*. Flies released into the mesh cages were selected based on when they emerged from western hemlock foliage collected from sites in the Pacific Northwest. In the field, after *La. nigrinus* prepupal drop, *Le. piniperda* adults emerge followed by an emergence of *La. argenticollis* adults, which can occur as *Le. piniperda* adults are still emerging (Dietschler et al. 2021). The majority of *Leucotaraxis* that were recovered from cages and confirmed to species were the western lineage of *Le. piniperda* and suggests that the majority of these flies were the offspring of the adults that were released into the treatments (Table 3). However, there were a large number of specimens (130 total specimens) that could not be identified to species due to contamination or low DNA presence. This could have been a result of how this study was designed. Cages used to hold *Le. piniperda* adults on a hemlock branch were applied in April to May and *Leucotaraxis* specimens were not collected from the cages until June to July and in September of the same year. Specimens may have been present within the cages for long periods of time and exposed to environmental conditions that likely caused fungal growth and degradation of DNA. Future field studies on *Le. piniperda* and *Le. argenticollis* that depend on molecular analyses should include frequent monitoring of sites for *Leucotaraxis* specimens throughout the study to prevent this.

In our study, we selected *Le. piniperda* instead of *Le. argenticollis* because *Le. piniperda* adult emergence occurs after *La. nigrinus* prepupal drop in the US Pacific Northwest (Dietschler et al. 2021). This enabled us to examine the impacts of *La. nigrinus* and *Le. piniperda* separately. However, the phenology of *Le. piniperda* in the eastern United States is unknown. Based on Dietschler et al. (2021), *Le. piniperda* adult emergence in the West occurs only once per year at the time when the progrediens generation is present. This led us to believe that *Le. piniperda* would be a good candidate for a caged branch study to test the impact on the HWA progrediens generation. However, our results suggest that *Le. piniperda* may not be as effective a biological control agent as *Le. argenticollis*. *Le. argenticollis* adult emergence occurs 1 or 2 times per year when the sistens generation is present and later when the progrediens generation is present (Dietschler et al. 2021). Another study that focused on the phenology of *Le. argenticollis* on caged branches in the eastern United States, found that *Le. argenticollis* adults emerged before and during the time when adult sistens were producing eggs and when progrediens nymphs and adults with eggs were present (Preston et al. 2023b). In addition, *Le. argenticollis* larvae were found throughout the year in NY and VA, with peaks occurring when HWA adults were producing eggs and when HWA nymphs of both generations were present (Preston et al. 2023b). This suggests that *Le. argenticollis* may have an impact on both generations of HWA, and with *La. nigrinus* already present at several locations in the eastern United States, may be more beneficial than *Le. piniperda* since there would be an additional predator targeting the sistens generation. While we cannot determine if *Le. piniperda* or *Le. argenticollis* would have more of an impact, we can confirm that *Le. piniperda* was able to reproduce at all field sites, which shows potential for establishment (Motley et al. 2017).

Impacts of Summer and Winter Conditions on HWA Populations

Several studies have indicated that HWA populations are susceptible to cold temperatures ≤–10 °C, with some variation on survival depending on geographic location (Parker et al. 1998, Skinner et al. 2003, Paradis et al. 2008, Trotter and Shields 2009, McAvoy et al. 2017). From our study, winter mortality ranged between 18.0% to 75.0% in 2021 and 5.0% to 60.0% in 2022. Although, we do not have temperature data, we can assume that the winter conditions in 2021 and 2022 were not detrimental to HWA populations throughout the eastern United States and did not result in suppressing HWA populations. For this study, we needed to apply mesh cages over hemlock branches to exclude *La. nigrinus* and to include *Le. piniperda* depending on the treatment. The proportion of HWA winter mortality slightly differed among treatments in 2021 and in 2022 (Fig. 2). Previous studies using mesh cages on eastern hemlock branches have found minimal variability in temperatures inside versus outside of the mesh cages (Preston et al. 2023a, 2023b). Therefore, we can assume that the effect of temperature was minimal on HWA population inside of the mesh cages.

No known previous studies have examined the impact of summer conditions on the progrediens generation of HWA. For our study, the number of dead, alive, and disturbed progrediens present on the 1- to 2-yr-old shoot growth on hemlock branches in June and July when adult progrediens had produced most of their eggs were recorded. From this, we determined that the percentage of dead progrediens, that was not caused by *Le. piniperda*, ranged between 9.6% to 79.0% in 2021 and 11.0% to 43.4% in 2022.There were no significant differences among treatments for progrediens summer

mortality, suggesting that cage effects were minimal across sites during the summer months for both years. We suggest that future studies should focus on the effect of summer conditions, such as high temperatures, drought conditions, or direct exposure to sunlight on the progrediens generation and determine how influential it may be to HWA population growth.

La. nigrinus and *Le. piniperda* Impacts to Eastern Hemlock Health

HWA feeding is known to cause multiple symptoms to eastern hemlock, such as premature needle drop, a reduction in new shoot growth, and branch dieback, which can eventually lead to tree mortality (McClure et al. 1996). For this study, we looked at the proportion of new shoot growth and the mean length of new shoot growth as indications of eastern hemlock health. We also tried to determine if the number of *Laricobius* spp. larvae and *Le. piniperda* specimens collected would correlate with these measurements. The results were inconclusive and presented a weak relationship between the presence of either predator and eastern hemlock health. One possible reason for this could be the short length of this study. A previous study that looked at the impact of *La. nigrinus* predation on HWA populations and on the net photosynthetic rate of eastern hemlock found a temporary effect where treatments with *La. nigrinus* had higher photosynthetic rates compared to treatments without *La. nigrinus* in June (Preston et al. 2023a). In addition, that study found that the treatment branches with a high density of *La. nigrinus* produced significantly more shoot growth than treatment branches without predators (Preston et al. 2023a), suggesting that *La. nigrinus* predation on sistens ovisacs had an indirect effect on eastern hemlock physiology. However, our study did not look at the photosynthetic rate, so we were unable to confirm if there were any physiological improvements of eastern hemlock health from the predator treatments. Another study, assessing the effect of an integrated pest management approach on eastern hemlock health, found that changes in eastern hemlock health lagged 0 to 3 yrs after HWA populations declined (Sumpter et al. 2018). Therefore, in our study we may have not been able to see the after effect from reducing HWA populations on treatment branches with predators, since our study evaluated eastern hemlock health less than 1 yr after predators effected HWA populations.

Another reason for these inconclusive results could be that the density of HWA sistens from previous years could have been more influential on the production of new shoot growth than the presence of the predators. Sussky and Elkinton (2014) found that mean new shoot growth length declined due to HWA densities that occurred the previous year. Therefore, new shoot production may be a function of both current HWA densities as well as the physiological impact of past HWA dynamics on the tree. And our study was not able to disentangle these short-term and long-term effects on hemlock growth. For predators to have an impact on eastern hemlock health, the predators may need to consistently suppress HWA populations for multiple years.

Cage Treatment Effects on Eastern Hemlock Health

For this study, 4 branches with low adelgid densities (<1 HWA/cm) were selected as cage treatments. Each cage treatment corresponded with one of the 4 treatments by when a cage was applied. For example, the Fall cage treatment corresponded with the No predator treatment, where cages were installed in the fall to prevent *La. nigrinus* from reaching the branches, and then, in the spring, cages were removed. These cage treatments were used to determine if there was a cage effect on eastern hemlock growth.

Overall, the percentage of new shoot growth on treatment branches, that started with >1 HWA/cm, ranged between 2.0% and 58.0%, and the percentage of new shoot growth on cage treatment branches ranged between 2.0% and 79.0%. The range of percentage of new shoot growth may be similar between predator treatments and cage treatments, however, for MD, NC, PA, and VA sites for both years, 3.4% and 68.4% of predator treatment replicates did not produce new shoot growth and 0.0% and 47.4% of the cage treatment replicates did not produce new shoot growth. This suggests that HWA densities >1 HWA/cm had a higher chance of not producing new shoot growth and that HWA densities at the beginning of the study could have influenced the percentage of new shoot growth, regardless of *La. nigrinus* and *Le. piniperda* predation that occurred on predator treatment branches. McClure (1991) suggested that the prohibition of new shoot growth of eastern hemlock occurred when more than 4 adelgid were found per cm of branch. However, McClure (1991) used only mature eastern hemlocks, examined only branches in the lower canopy, and excluded the effect of environmental factors, so the suggested damage threshold of eastern hemlock should be considered with caution. Based on the results here, it suggests that HWA densities from the previous year that were >1 HWA/cm seem to reduce new shoot growth the following year, but it is difficult to know what the exact threshold would be.

Acknowledgements

We would like to thank Tonya Bittner for aiding in the collection and shipping of *Le. piniperda* adults to our field sites. We would like to thank undergraduates Ryan Anderson, Anna Jurisik, Haley Olsen-Hodges, Maggie Smith, Liam Williams, Larkin Rae, and Rachel Hoffman for their help with *Laricobius* larvae collection and branch dissections. We would also like to thank Tom McAvoy, Ryan Mays, Carrie Jubb, Ashleigh Hillen, Olivia Andrews, Biff Thompson, Patrick Simons, and David Bechtel for their help with field site set up and branch collection. We would like to thank Young Ho Yun, Anbin Rhee, and Tsering Dolkar for consultation on the statistical analyses.

Author Contributions

Carrie E. Preston (Conceptualization [lead], Data curation [lead], Formal analysis [lead], Investigation [lead], Methodology [lead], Project administration [equal], Supervision [equal], Validation [equal], Visualization [lead], Writing—original draft [lead], Writing—review & editing [lead]), Timothy Shively (Formal analysis [equal], Visualization [supporting], Writing—review & editing [supporting]), Mark C. Whitmore (Conceptualization [equal], Methodology [equal], Resources [lead], Writing—review & editing [supporting]), Jerome F. Grant (Conceptualization [supporting], Data curation [equal], Investigation [supporting], Methodology [supporting], Writing—review & editing [supporting]), Albert Mayfield (Data curation [equal], Investigation [supporting], Methodology [supporting], Writing—review & editing [equal]), Timothy Tomon

(Data curation [supporting], Investigation [supporting], Writing—review & editing [supporting]), Nathan P. Havill (Investigation [equal], Methodology [equal], Resources [supporting], Writing—review & editing [equal]), Zephyr Zembrzuski (Investigation [supporting], Methodology [supporting], Resources [supporting], Writing—review & editing [supporting]), John Seiler (Conceptualization [supporting], Methodology [supporting], Writing—review & editing [equal]), and Scott M. Salom (Conceptualization [equal], Formal analysis [supporting], Funding acquisition [lead], Investigation [supporting], Methodology [supporting], Project administration [equal], Resources [lead], Supervision [lead], Validation [equal], Visualization [supporting], Writing—review & editing [equal])

Supplementary Material

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

Funding

This work was supported by USDA Forest Service grant number 19DG11083150009.

Conflicts of Interest

None declared.

Data Availability

The data underlying this article were provided by Virginia Tech under license / by permission. Data will be shared on request to the corresponding author with permission of Virginia Tech.

References

Brooks ME, Kristensen K, van Benthem KJ, et al. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *RJ*. 9:378–400. https://doi.org/10.32614/RJ-2017-066

Crandall RS, Chandler JL, Dietschler NJ, et al. 2023. Establishment and postrelease recovery of *Laricobius nigrinus* and *Laricobius osakensis* (Coleoptera: Derodontidae), released for biological control of *Adelges tsugae* (Hemiptera: Adelgidae), in the Northeastern United States. *Environ. Entomol.* 52:769–778. https://doi.org/10.1093/ee/nvad073

Crandall RS, Jubb CS, Mayfield AE, et al. 2020. Rebound of *Adelges tsugae* spring generation following predation on overwintering generation ovisacs by the introduced predator *Laricobius nigrinus* in the eastern United States. *Biol. Con.* 145:104264. https://doi.org/10.1016/j.biocontrol.2020.104264

Dietschler N, Bittner T, Devine N, et al. 2023. Overwintering diapause and survival of western *Leucotaraxis argenticollis*, a promising biological control agent for *Adelges tsugae*, in the eastern United States. *Biol. Con.* 182:105233. https://doi.org/10.1016/j.biocontrol.2023.105233

Dietschler NJ, Bittner TD, Trotter RT, et al. 2021. Biological control of hemlock woolly adelgid: implications of adult emergence patterns of two *Leucopis* spp. (Diptera: Chamaemyiidae) and *Laricobius nigrinus* (Coleoptera: Derodontidae) larval drop. *Environ. Entomol.* 50:803–813. https://doi.org/10.1093/ee/nvab037

Dilling C, Lambdin P, Grant J, et al. 2009. Community response of insects associated with eastern hemlock to imidacloprid and horticultural oil treatments. *Environ. Entomol.* 38:53–66. https://doi.org/10.1603/022.038.0108

Dodd D. 2023. HWA distribution maps. http://hiro.ento.vt.edu/hwa/index.php/distribution-maps/

Domec JC, Rivera LN, King JS, et al. 2013. Hemlock woolly adelgid (*Adelges tsugae*) infestation affects water and carbon relations of eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*). *New Phytol.* 199:452–463. http://doi.org/10.1111/nph.12263

Ellison AM. 2014. Experiments are revealing a foundation species: a case study of eastern hemlock (*Tsuga canadensis*). *Adv. Ecol.* 2014:456904. http://dx.doi.org/10.1155/2014/456904

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

Farjon A. 1990. *Pinaceae: drawings and descriptions of the genera Abies, Cedrus, Pseudolarix, Keteleeria, Nothotsuga, Tsuga, Cathaya, Pseudotsuga, Larix and Picea*. Koeltz Scientific Books.

Foley JR, Jubb CS, Cole DA, et al. 2021. Historic assessment and analysis of the mass production of *Laricobius* spp. (Coleoptera: Derodontidae), biological control agents for the hemlock woolly adelgid, at Virginia Tech. *J. Insect Sci.* 21:1–12. http://doi.org/10.1093/jisesa/ieab005

Gaimari S, Havill N. 2021. A new genus of Chamaemyiidae (Diptera: Lauxanioidea) predaceous on Adelgidae (Hemiptera), with a key to chamaemyiid species associated with Pinaceae. *Zootaxa* 5067:1–39. https://doi.org/10.11646/zootaxa.5067.1.1

Godman RM, Lancaster K. 1990. *Tsuga canadensis* (L.) Carr., eastern hemlock. In: Burns, R, Honkala BH, editors. *Silvics of North America: 1. Conifers. Agriculture Handbook 654*. USDA Forest Service. p. 604–612.

Grubin SM, Ross DW, Wallin KF. 2011. Prey suitability and phenology of *Leucopis* spp. (Diptera: Chamaemyiidae) associated with hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. *Environ. Entomol.* 40:1410–1416. http://dx.doi.org/10.1603/EN11127

Hartig F. 2022. *DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models*. R package version 0.4.7. Vienna (Austria): The R Foundation for Statistical Computing; Comprehensive R Archive Network (CRAN). https://CRAN.R-project.org/package=DHARMa

Havill N, Shiyake S, Lamb Galloway A, et al. 2016. Ancient and modern colonization of North America by hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), an invasive insect from East Asia. *Mol. Ecol.* 25:2065–2080. https://doi.org/10.1111/mec.13589

Havill NP, Bittner TD, Andersen JC, et al. 2023. Prey-associated genetic differentiation in two species of silver fly (Diptera: Chamaemyiidae), *Leucotaraxis argenticollis* and *L. piniperda*. *Insect Syst. Div.* 7:1–16. https://doi.org/10.1093/isd/ixad007

Havill NP, Gaimari SD, Caccone A. 2018. Cryptic east-west divergence and molecular diagnostics for two species of silver flies (Diptera: Chamaemyiidae: *Leucopis*) from North America being evaluated for biological control of hemlock woolly adelgid. *Biol. Con.* 121:23–29. https://doi.org/10.1016/j.biocontrol.2018.02.004

Havill NP, Montgomery ME. 2008. The role of arboreta in studying the evolution of host resistance to the hemlock woolly adelgid. *Arnoldia* 65:2–9. https://doi.org/10.5962/p.251017

Hebert PDN, Penton EH, Burns JM, et al. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proc. Natl. Acad. Sci. USA*. 101:14812–14817. https://doi.org/10.1073/pnas.0406166101

Huggett B, Savage J, Hao G, et al. 2018. Impact of hemlock woolly adelgid (*Adelges tsugae*) infestation on xylem structure and function and leaf physiology in eastern hemlock (*Tsuga canadensis*). *Funct. Plant Biol.* 45:501–508. https://doi.org/10.1071/FP17233

Jubb CS, Heminger AR, Mayfield AE, et al. 2020. Impact of the introduced predator, *Laricobius nigrinus*, on ovisacs of the overwintering

generation of hemlock woolly adelgid in the eastern United States. *Biol. Con.* 143:104180. https://doi.org/10.1016/j.biocontrol.2019.104180

Kohler G, Wallin K, Ross DW. 2016. Seasonal phenology and abundance of *Leucopis argenticollis*, *Leucopis piniperda* (Diptera: Chamaemyiidae), *Laricobius nigrinus* (Coleoptera: Deridontidae) and *Adelges tsugae* (Hemiptera: Adelgidae) in the Pacific Northwest USA. *Bull. Entomol. Res.* 106:546–550. https://doi.org/10.1017/S0007485316000250

Kohler GR, Stiefel VL, Wallin KF, et al. 2008. Predators associated with the hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. *Environ. Entomol.* 37:494–504. https://doi.org/10.1093/ee/37.2.494

Korhonen P, Hui FK, Niku J, et al. 2024. A comparison of joint species distribution models for percent cover data. *Methods Ecol. Evol.* 15:2359–2372. https://doi.org/10.1111/2041-210X.14437

Kubinec R. 2023. Ordered beta regression: a parsimonious, well-fitting model for continuous data with lower and upper bounds. *Polit. Anal.* 31:519–536. https://doi.org/10.1017/pan.2022.20

Lenth RV, Piaskowski J. 2026. *emmeans: Estimated marginal means, aka least-squares means*. R package version 2.0.2. Vienna (Austria): The R Foundation for Statistical Computing; Comprehensive R Archive Network (CRAN). https://CRAN.R-project.org/package=emmeans

Limbu S, Keena M, Whitmore M. 2018. Hemlock woolly adelgid (Hemiptera: Adelgidae): a non-native pest of hemlocks in eastern North America. *J. Integr. Pest Manag.* 9:27. https://doi.org/10.1093/jipm/pmy018

Mausel DL, Salom SM, Kok LT, et al. 2008. Propagation, synchrony, and impact of introduced and native *Laricobius* spp. (Coleoptera: Derodontidae) on hemlock woolly adelgid in Virginia. *Environ. Entomol.* 37:1498–1507. https://doi.org/10.1603/0046-225X-37.6.1498

Mausel DL, Salom SM, Kok LT, et al. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the eastern United States. *Environ. Entomol.* 39:440–448. https://doi.org/10.1603/EN09088

Mayfield AE, Bittner TD, Dietschler NJ, et al. 2023. Biological control of hemlock woolly adelgid in North America: history, status and outlook. *Biol. Con.* 185:105308. https://doi.org/10.1016/j.biocontrol.2023.105308

McAvoy TJ, Régnière J, St-Amant R, et al. 2017. Mortality and recovery of hemlock woolly adelgid (*Adelges tsugae*) in response to winter temperatures and predictions for the future. *Forests* 8:497. https://doi.org/10.3390/f8120497

McClure MS. 1989. Evidence of a polymorphic life cycle in the hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). *Ann. Entomol. Soc. Am.* 82:50–54. https://doi.org/10.1093/aesa/82.1.50

McClure MS. 1991. Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. *Environ. Entomol.* 20:258–264.

McClure M, Salom S, Shields K. 1996. *Hemlock woolly adelgid*. Forest Health Technology Enterprise Team.

McDonald KM, Seiler JR, Wang B, et al. 2023. Effects of hemlock woolly adelgid control using imidacloprid on leaf-level physiology of eastern hemlock. *Forests* 14:1228. https://doi.org/10.3390/f14061228

Motley K, Havill NP, Arsenault-Benoit AL, et al. 2017. Feeding by *Leucopis argenticollis* and *Leucopis piniperda* (Diptera: Chamaemyiidae) from the western USA on *Adelges tsugae* (Hemiptera: Adelgidae) in the eastern USA. *Bull. Entomol. Res.* 107:699–704. https://doi.org/10.1017/S0007485317000219

Nelson LA, Dillaway DN, Rieske LK. 2014. Effect of an exotic herbivore, *Adelges tsugae*, on photosynthesis of a highly susceptible *Tsuga* host, with notes on conspecifics. *Arthropod. Plant Interact.* 8:9–15. https://doi.org/10.1007/s11829-013-9285-9

Paradis A, Elkinton J, Hayhoe K, et al. 2008. Role of winter temperature and climate change on the survival and future range expansion of the hemlock woolly adelgid (*Adelges tsugae*) in eastern North America. *Mitig. Adapt. Strateg. Glob. Change* 13:541–554. https://doi.org/10.1007/s11027-007-9127-0

Parker BL, Skinner M, Gouli S, et al. 1998. Survival of hemlock woolly adelgid (Homoptera: Adelgidae) at low temperatures. *For. Sci.* 44:414–420. https://doi.org/10.1093/forestscience/44.3.414

Preston CE, Arneson A, Seiler JR, et al. 2023a. The impact of predation of *Laricobius nigrinus* (Coleoptera: Derodontidae) on *Adelges tsugae* (Hemiptera: Adelgidae) and *Tsuga canadensis* (Pinales: Pinaceae) tree health. *Forests* 14:698. https://doi.org/10.3390/f14040698

Preston CE, Dietschler NJ, Whitmore MC, et al. 2023b. Phenology of *Leucotaraxis argenticollis*, a specialist predator of the invasive hemlock woolly adelgid, in the eastern US. *Environ. Entomol.* 52:1008–1019. https://doi.org/10.1093/ee/nvad103

R Core Team. 2025. R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org

Rigby RA, Stasinopoulos DM. 2005. Generalized Additive Models for Location, Scale and Shape. *J. R. Stat. Soc. Ser. C Appl. Stat.* 54:507–554. https://doi.org/10.1111/j.1467-9876.2005.00510.x

Rose A, Ross D, Havill N, et al. 2020. Coexistence of three specialist predators of the hemlock woolly adelgid in the Pacific Northwest USA. *Bull. Entomol. Res.* 110:303–308. https://doi.org/10.1017/S0007485319000622. https://doi.org/10.1017/S0007485319000622

Salom S, Kok L, Lamb A, et al. 2012. Laboratory rearing of *Laricobius nigrinus* (Coleoptera: Derodontidae): a predator of the hemlock woolly adelgid (Hemiptera: Adelgidae). *Psyche (Stuttg)* 2012:1–9. https://doi.org/10.1155/2012/936519

Schomaker ME, Zarnoch SJ, Bechtold WA, et al. 2007. Crown-condition classification: a guide to data collection and analysis; General Technical Report SRS-102; USDA Forest Service.

Skinner M, Parker BL, Gouli S, et al. 2003. Regional responses of hemlock woolly adelgid (Homoptera: Adelgidae) to low temperatures. *Environ. Entomol.* 32:523–528. https://doi.org/10.1603/0046-225X-32.3.523

Stoetzel MB. 2002. History of the introduction of *Adelges tsugae* based on voucher specimens in the Smithsonian Institute National Collection of Insects. In: Onken B, Reardon R, and Lashomb J, editors. *Hemlock woolly adelgid in the eastern United States symposium*. U.S. Department of Agriculture Forest Service and State University of NJ Rutgers. p. 12.

Sumpter KL, McAvoy TJ, Brewster CC, et al. 2018. Assessing an integrated biological and chemical control strategy for managing hemlock woolly adelgid in southern Appalachian forests. *For. Ecol. Manage.* 411:12–19. https://doi.org/10.1016/j.foreco.2018.01.018

Sussky EM, Elkinton JS. 2014. Density-dependent survival and fecundity of hemlock woolly adelgid (Hemiptera: Adelgidae). *Environ. Entomol.* 43:1157–1167. https://doi.org/10.1603/EN13356

Toland A, Brewster C, Mooneyham K, et al. 2018. First report on establishment of *Laricobius osakensis* (Coleoptera: Derodontidae), a biological control agent for hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), in the eastern US. *Forests* 9:496. https://doi.org/10.3390/f9080496

Trotter RT, Shields KS. 2009. Variation in winter survival of the invasive hemlock woolly adelgid (Hemiptera: Adelgidae) across the eastern United States. *Environ. Entomol.* 38:577–587. https://doi.org/10.1603/022.038.0309

Vieira LC, Salom SM, Kok LT. 2012. Functional and numerical response of *Laricobius* spp. predators (Coleoptera: Derodontidae) on hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae). *Biol. Con.* 61:47–54. https://doi.org/10.1016/j.biocontrol.2012.01.006

Wallace MS, Hain FP. 2000. Field surveys and evaluation of native and established predators of the hemlock woolly adelgid (Homoptera: Adelgidae) in the southeastern United States. *Environ. Entomol.* 29:638–644. https://doi.org/10.1603/0046-225X-29.3.638

Wickham H. 2016. *ggplot2: Elegant graphics for data analysis*. Springer.

Wickham H, François R, Henry L, et al. 2023. *dplyr: A grammar of data manipulation*. R package version 1.1.4. Vienna (Austria): The R Foundation for Statistical Computing; Comprehensive R Archive Network (CRAN). https://CRAN.R-project.org/package=dplyr.

Journal of Economic Entomology 2026 00(00) 1–12
https://doi.org/10.1093/jee/toag202
Research Biological and Microbial Control