Environmental Entomology, 49(4), 2020, 823–828
doi: 10.1093/ee/nvaa049
Advance Access Publication Date: 28 May 2020
Research

Community Ecology

Temporal Asynchrony of Adult Emergence Between *Leucopis argenticollis* and *Leucopis piniperda* (Diptera: Chamaemyiidae), Predators of the Hemlock Woolly Adelgid (Hemiptera: Adelgidae), with Implications for Biological Control

Alex N. Neidermeier,[1,5] Darrell W. Ross, [2] Nathan P. Havill, [3] and Kimberly F. Wallin[4]

[1]Rubenstein School of Environment and Natural Resources, The University of Vermont, Burlington, VT 05405,[2]Department of Entomology, School of Natural Resource Sciences, College of Agriculture, Food Systems, and Natural Resources, North Dakota State University, Fargo, ND 58105,[3]USDA Forest Service, Northern Research Station, Hamden, CT 06514,[4]College of Science and Mathematics, North Dakota State University, Fargo, ND 58105, and [5]Corresponding author, e-mail: anneiderm@gmail.com

Subject Editor: Jason Schmidt

Received 24 October 2019; Editorial decision 7 April 2020

Abstract

Two species of silver fly, *Leucopis argenticollis* (Zetterstedt) and *Leucopis piniperda* (Malloch) (Diptera: Chamaemyiidae), from the Pacific Northwest region of North America have been identified as potential biological control agents of hemlock woolly adelgid (Hemiptera: Adelgidae: *Adelges tsugae* Annand) in eastern North America. The two predators are collectively synchronized with *A. tsugae* development. To determine whether adult emergence of the two species of silver fly are also synchronized with one another, we collected adult *Leucopis* which emerged from *A. tsugae*-infested western hemlock [Pinaceae: *Tsuga heterophylla* (Raf.) Sarg.] from four sites in the Pacific Northwest over a 29-d period. Specimens were collected twice daily in the laboratory and identified to species using DNA barcoding. The study found that more adult *Leucopis* were collected in the evening than the morning. Additionally, the daily emergences of adults over the 29-d sampling period exhibited sinusoidal-like fluctuations of peak abundance of each species, lending evidence to a pattern of temporal partitioning. This pattern could have logistical implications for their use as biological control agents in eastern North America, namely the need to release both species for maximum efficacy in decreasing *A. tsugae* populations.

Key words: resource partitioning, biological control, temporal partitioning

Two species of silver fly (Diptera: Chamaemyiidae), *Leucopis argenticollis* (Zetterstedt) and *Leucopis piniperda* (Malloch) [misidentified as *Leucopis atrifacies* Aldrich in Kohler et al. (2008), see Grubin et al. (2011)], from the Pacific Northwest show promise as biological control agents for hemlock woolly adelgid (*Adelges tsugae* Annand) in eastern North America. *Adelges tsugae* were first observed in the eastern United States almost 70 yr ago and were likely introduced from Japan on ornamental trees near Richmond, Virginia (Stoetzel 2002, Havill et al. 2014). *Adelges tsugae* has spread throughout most of the range of eastern and Carolina hemlocks (*Tsuga canadensis* (L.) Carriére and *T. caroliniana* Engelmann, respectively), incurring aesthetic, ecological, and economic impacts (Quimby 1996). Damages to hemlocks caused by *A. tsugae* include needle drop, desiccation, branch dieback, nutrient loss, and death (McClure 1991). At the landscape scale, the loss of hemlock trees changes forest composition (Orwig et al. 2002, Ellison et al. 2005, Brantley et al. 2013) and increases stream temperatures, soil pH, and nitrification (Jenkins et al. 1999, Kizlinski et al. 2002). The effects of hemlock loss also negatively impact the abundance of several species of fish and bird found uniquely in association with hemlock ecosystems (Ross et al. 2003, Angelini et al. 2011).

Adelges tsugae has multiple native genetic lineages in Asia and western North America (Havill et al. 2018). Potential biological control agents from these regions have been evaluated based on their host specificity (e.g., Zilahi-Balogh et al. 2002, Grubin et al. 2011), synchronization with *A. tsugae* development (Grubin et al. 2011), long-term potential to establish (Mausel et al. 2010) and effectiveness at lowering *A. tsugae* populations (Mausel et al. 2008, Vose et al. 2013). In North America, *A. tsugae* is parthenogenetic, with two generations, sistens and progrediens, which oviposit eggs into ovisacs on hemlock branches in approximately March and June, respectively (McClure 1987).

Surveys of *A. tsugae* predators in the West revealed that two species of silver fly, *Leucopis argenticollis* and *Leucopis piniperda*, were collectively the most abundant predators associated with *A. tsugae* infested western hemlocks (Kohler et al. 2008, 2016). Research published by Grubin et al. (2011) documented the incidence of the four life stages (egg, larva, puparium, and adult) for *Leucopis* spp., but the total lifespan of *Leucopis* spp. could not be discerned from these data. The larval stage, which was documented feeding on adelgids (Grubin et al. 2011, Motley et al. 2017), were collected on all but 3 d of the year-long study conducted by Grubin et al. (2011), with peak abundances in June and July. This study also found that *Leucopis* spp. abundance was positively correlated with each of the two *A. tsugae* generations in the western United States, a possible indicator of life cycle synchronicity.

Remarkably, *Leucopis argenticollis* and *L. piniperda* are also found in eastern North America (McAlpine and Tanasijtshuk 1972), but eastern populations of both species are genetically divergent from their western relatives (Havill et al. 2018). The eastern flies predate on pine adelgids (*Pineus* spp.) and have not been collected in the east on *A. tsugae* (Havill et al. 2018). *Leucopis* spp. are promising candidates for biological control due to their abundance on *A. tsugae* in the Pacific Northwest (Kohler et al. 2007, Kohler et al. 2016), association with both *A. tsugae* generations (Kohler et al. 2008, Grubin et al. 2011), host-specificity on adelgid eggs and nymphs (Grubin et al. 2011), and successful survival and reproduction in enclosed-release experiments in Tennessee, New York (Motley et al. 2017), and North Carolina (experiments on going). Other species of Chamaemyiidae have successfully controlled adelgid species in Hawaii, Chile, and Africa (Zúñiga 1985, Allo and Karanja 1986, Culliney et al. 1988, Greathead 1995), suggesting that this group of predators could be effective against *A. tsugae*.

However, biological control programs using other species of Chamaemyiidae have encountered issues with predator–prey phenological synchronization (Mitchell and Wright 1967, Gaimari 1991). Phenological assessment of *L. piniperda* and *L. argenticollis* from the Pacific Northwest has been limited. Synchronization of these two species of *Leucopis* was initially studied in aggregate because it is difficult to distinguish them from one another based on morphology. Collectively, the two species were shown to predate both the sistens and progrediens generations of the adelgid's yearly life cycle in the Pacific Northwest (Kohler et al. 2008, Grubin et al. 2011). However, this left a gap in knowledge about how the two species of fly coexist at collection sites in the western United States. Specimens can now be reliably identified to species using DNA barcoding (Havill et al. 2018, Rose et al. 2019), allowing each species to be studied separately. Using these DNA barcoding methods, Rose et al. (2019) did not find evidence of spatial niche differentiation between the two fly species at four sites in the Pacific Northwest. In the same study, bi-weekly sampling indicated no obvious patterns of temporal differentiation between the two species. However, the temporally coarse sampling of flies in the study by Rose et al. (2019) may have masked finer scale patterns.

Therefore, our research focused on assessing other avenues of differentiating niche partitioning between the two species of *Leucopis* in the West in three parts. First, temporal dynamics were explored through a semidiurnal, daily assessment of adult emergence (hereafter referred to as AM/PM for simplicity). Second, aggregate daily abundances were assessed over a 29-d timeframe to determine whether emergence patterns of each species may be evident over the course of weeks instead of days. Finally, the effect of collection site was assessed to determine whether it may have a statistical effect on each species adult emergence. The results collectively represent a novel, finer-scale temporal survey of *Leucopis* dynamics in the Pacific Northwest. This approach avoids aggregate sampling, which could potentially confound crepuscular emergence, daily emergence, and effects of site. The clarification of temporal differentiation may help optimize the sampling of each *Leucopis* species from the West. If this strategy is predicted to have the best probability of decreasing *A. tsugae* populations, this will allow both species of *Leucopis* to be optimally released in the East.

Materials and Methods

Field Methods

To explore *Leucopis* spp. temporal dynamics, we assessed adult emergence of *Leucopis* spp. in the lab using *A. tsugae* -infested *Tsuga heterophylla* (Raf.) Sarg. foliage collected in the Pacific Northwest. Sites in Washington and Oregon with abundant *A. tsugae* (approximately 50 ovisacs per 30 cm) were first identified. Nine sites were found to have suitably high populations of *A. tsugae*, and thus were likely able to support ample numbers of *Leucopis* spp. to achieve the study's goals (Supp Table 1 [online only]). At each site, foliage was initially collected between 1000 and 1600, between 30 March and 7 April 2019. Foliage was transported to Oregon State University (OSU), Department of Forest Ecosystems and Society, Corvallis, OR, and placed in bug dorms (Item number BD2120, MegaView Science, Taiwan; referred to as 'cages' hereafter). Each cage was filled with as much foliage as could reasonably fit into the space. The cages also contained two Sterilite plastic shoeboxes (31 × 19 × 10 mm) with floral foam blocks, saturated with deionized water, into which branch clippings were inserted with a small amount of water at the bottom of each shoebox to maintain turgidity of the cut branches. Cages were kept in a laboratory at room temperature (20–25°C). In order to establish an ample dataset from which to subsample specimens, four sites with the most adult *Leucopis* emergence were chosen for this analysis. For these four sites, Grant Park, Point Defiance, Thurston Title Co., and Tumwater Falls (Supp Table 2 [online only]), at least 60 adult *Leucopis* emerged in the lab within 2 wk of the initial collection. Additional foliage was, therefore, collected from these four respective sites on April 29th and 13 May 2019 and placed in separate cages.

Each cage was inspected for adult *Leucopis* in the morning (between 730 and 900) and evening (between 1830 and 2000). *Leucopis* from each cage were aspirated and placed into vials with 95% EtOH. For each vial, whether adults emerged in the morning or evening (AM/PM), the date, and collection site were recorded. This procedure was carried out for all but 2 d (JD 117 and 118) of the full study period (JD 106–135), when collection was not possible. Vials were stored at –20°C until overnight shipment to the USDA Forest Service George D. Aiken Forestry Sciences Laboratory at the University of Vermont (UVM) in Burlington, VT, where samples were immediately placed into –10°C storage until DNA extraction and identification using DNA barcoding.

A subsample from the total 3,808 collected specimens was selected for analyses. Up to six adult flies, as available, were identified to species using DNA-barcoding per AM/PM, laboratory collection date, and site, resulting in a total of 767 individuals. This methodology provided ample data for a comparison of proportions of collected adult specimens to fulfill the spatial and temporal objectives of the study.

DNA Barcoding

Leucopis argenticollis is morphologically distinguished from *L. piniperda* by the patterns of postpronotal setae, which can be difficult to observe. Therefore, species was determined using DNA barcoding methods outlined in Havill et al. (2018). Briefly, each fly was punctured with a sterilized insect pin to expose contents of the body cavity and incubated with proteinase K at 56°C for a minimum of 1 h. After incubation, the fly cuticles were recovered as morphological vouchers and deposited at the Yale Peabody Museum of Natural History with accession numbers ENT961561 through ENT961676. DNA was extracted from the remaining liquid using the Mag-Bind Blood and Tissue Kit (Omega Bio-Tek, Norcross, GA) using the manufacturer's protocol. The standard 658 bp DNA barcoding portion on the 5′ end of the mitochondrial cytochrome oxidase I gene was amplified and sequenced using primers LepF1 and LepR1 (Hebert et al. 2004). DNA sequences were compared to those of known specimens (described in Havill et al. 2018, and available on GenBank) for species identification.

Statistical Analyses

All data sets were tested for normality using the Shapiro–Wilk test. Mean daily abundances were calculated for the collected subset of 767 DNA-barcoded *Leucopis* for each species at each AM/PM interval and site. Differences in species abundances in the morning versus evening emergence were assessed using Welch's Two-Sample *t*-test with a null hypothesis of no difference between means. Both *L. piniperda* and *L. argenticollis* daily abundance data were not normally distributed (W = 0.92; P = 0.03 and W = 0.81; P = 0.0001, respectively). Accordingly, a Spearman rank correlation test was applied to the two species' abundances over the 29-d study period. The effect of site on abundance of each species was assessed using one-way analyses of variance. All data analyses were run in R Studio version 3.5.2 (RCoreTeam 2018).

Results

Results Overview

Our study found differences in the abundance of each species of *Leucopis* collected in the laboratory over a 29-d collection period. Of the 767 adult flies, there was a higher proportion of *L. piniperda* (71.1%) than *L. argenticollis* (28.9%) (t = 3.35, df = 44.19, P = 0.001). In addition, four flies that emerged from foliage collected at the Grant Park site were identified as a different species of Chamaemyiidae, *Neoleucopis atratula* (Ratzeburg).

Temporal Patterns

Our study first assessed temporal dynamics of the subsample of DNA-identified, emerged adults of *Leucopis* spp. collected in the lab. Collectively, we found that *Leucopis* emergence was higher at the PM collection (59.2%) than the AM collection (40.8%) (t = –2.66; df = 52.84; P = 0.01) (Fig. 1). However, when evaluated by species, we found no effect of time of day for *L. argenticollis* (t = –0.95; df = 51.69; P = 0.35) or *L. piniperda* (t = –1.61; df = 51.18; P = 0.11).

Within the 29-d study period, the abundances of *L. argenticollis* and *L. piniperda* appeared to vary in a sinusoidal pattern (Fig. 2), with *L. piniperda* emerging before *L. argenticollis*, and peaks of abundance alternating between species. Peak totals of *L. piniperda* occurred on Julian Date (JD) 106 (49 adults), JD 127 (43 adults), and JD 134 (25 adults). Peak totals of *L. argenticollis* occurred on JD 110 (19 adults) and JD 133 (28 adults). *L. piniperda* were not found (i.e., abundance of zero) between JD 113 and JD 116 while no *L. argenticollis* were found between JD 106 and JD 109 or JD 121 and JD 129. The number of *L. piniperda* and *L. argenticollis* were found to be inversely correlated over the study period (rho = –0.71; S = 6263; P < 0.0001).

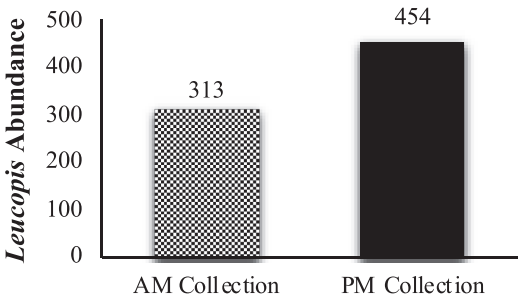

Fig. 1. Total number of adult *Leucopis* collected from laboratory cages in the morning and evening pooled by foliage collection sites and day of collection in the laboratory. Emergence was found to be higher at the PM collection (59.2% of sampled adult flies) than the AM collection (40.8% of sampled adult flies) (t = –2.66; df = 52.84; P = 0.01).

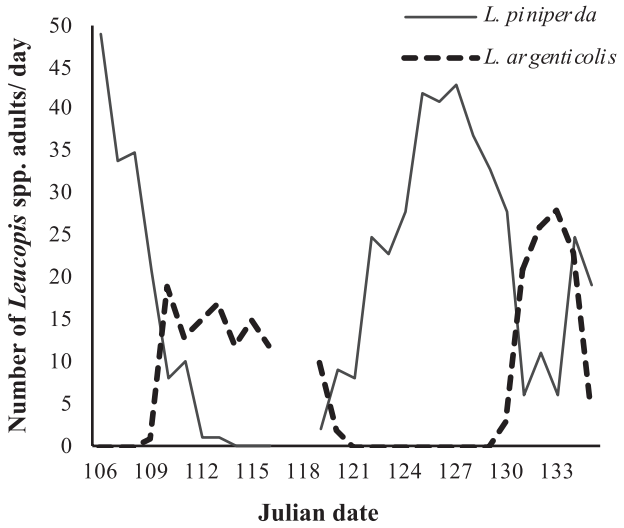

Fig. 2. Daily total number of *Leucopis piniperda* and *L. argenticollis* adults collected from laboratory cages with *Adelges tsugae*-infested *Tsuga heterophylla* foliage from four sites. Data were collected between 16 April and 15 May 2019 (JD 106–135). No data were collected on Julian dates 117 and 118.

Site Effects on Species' Abundance Patterns

Leucopis spp. emerged from branches collected at all sites. The greatest total of emerged *Leucopis* adults were collected from, in descending order, Point Defiance (1,481 collected, 234 sampled), Thurston Title Co. (781 collected, 194 sampled), Tumwater Falls (925 collected, 192 sampled), and Grant Park (526 collected, 147 sampled) (Fig. 3a). The number of *L. argenticollis* did not vary statistically among sites (F = 0.822; df = 3, 48; P = 0.488), however, the mean number of *L. piniperda* did vary statistically by site, specifically Thurston Title Co., which has comparably higher variability for this species: Point Defiance (4.94 ± SE 0.38), Thurston Title Co. (5.03 ± SE 0.28), Tumwater Falls (4.24 ± SE 0.46), and Grant Park (3.38 ± SE 0.39) (Fig. 3b; F = 3.828; df = 3, 118; P = 0.0117).

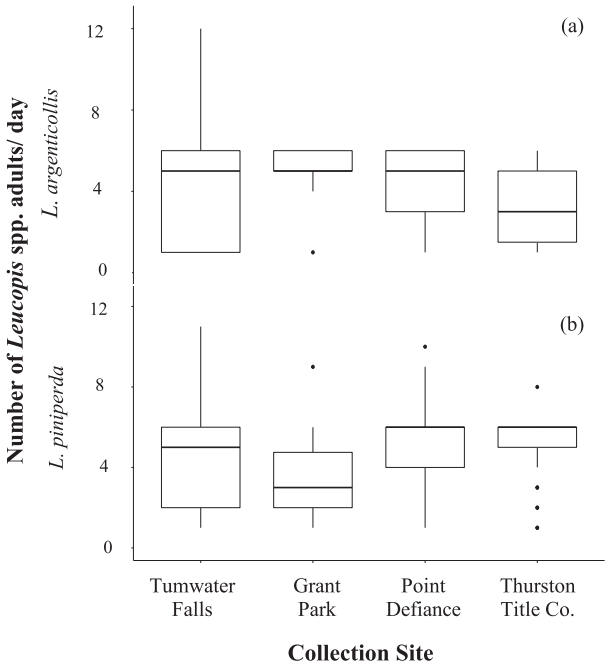

Fig. 3. Median number of (a) *L. argenticollis* and (b) *L. piniperda* adults collected per day from cages between 16 April and 15 May 2019 (JD 106–135) is represented by the central, bold line in each box plot below. Dots represent statistical outliers at each site. *Adelges tsugae*-infested foliage from which flies emerged was collected from Tumwater Falls, Point Defiance, and Thurston Title Co. in Washington and Grant Park in Oregon.

Discussion

Our results provide insight on the emergence times of *L. argenticollis* and *L. piniperda* from the Pacific Northwest which may improve their implementation as biological control agents of *A. tsugae* in eastern North America. Overall, we found that *L. piniperda* outnumbered *L. argenticollis* by 42.2%. This is seemingly at odds with the findings of Kohler et al. (2008), Grubin et al. (2011), and Rose et al. (2019), which all found higher abundances of *L. argenticollis*. However, unlike previous studies, we examined *Leucopis* spp. populations at a finer-scale (daily) over a relatively short time-period (29 d). Additionally, the sample size used in the current study was nearly seven times (n = 767 *Leucopis* spp.) that of previous studies (Kohler et al. 2008, n = 99, Grubin et al. 2011, n = 125, Rose et al. 2019, n = 76). In terms of within-day temporal patterns, our results indicate that both species of *Leucopis* combined had marginally greater adult emergence at the evening collection time across all sites (Fig. 1). This synchronous daily activity may be useful information for collection of *Leucopis* spp. in the West and for timing its release on *A. tsugae* in the East.

The recovery of a few *Neoleucopis atratula* specimens among the *Leucopis* feeding on *A. tsugae* in our study represents a new prey record. *Neoleucopis atratula* is a European species that was introduced to New Brunswick, Canada from Germany in 1965 for biological control of *Adelges piceae* (Ratzeburg), an invasive pest of fir trees (*Abies*) in North America (Schooley et al. 1981). In addition to being established in the East, *Neoleucopis atratula* has been recovered feeding on *Adelges piceae* in the Pacific Northwest in British Columbia in 1990–1991 (Humble 1994), but it is not known how it arrived on the west coast. This species primarily feeds on *A. piceae* and related adelgid species on *Abies* but has also been reported on *Pineus* species on *Pinus* (McAlpine and Tanasijtshuk 1972). Similar to Greathead (1995), who concluded that *N. atratula* would not be an effective control for *Pineus* sp. in Africa, *N. atratula* probably would not contribute to *A. tsugae* control because it primarily feeds on *A. piceae*.

In terms of longer-term temporal dynamics, patterns over a 29-d period demonstrated an inverse relationship between the abundances of each species' daily emerged adults which followed a sinusoidal pattern with peaks of abundance alternating between species. Although examining the drivers for this pattern is outside the scope of this study, alternating abundances could result in decreased interspecific competition (Walter 1991). The segregation of adult emergence could provide an opportunity for the predatory larval stages of each *Leucopis* species to be consequently staggered (Pellmyr 1989, Pompanon et al. 2006). This pattern could also be a residual effect of prey availability (Grubin et al. 2011, Ximenez-Embun et al. 2014), or of the specialization of each species of larval stage *Leucopis* in predating specific parts of *A. tsugae's* life cycle (Pellmyr 1989, Pampanon et al. 2006).

The complementary sinusoidal patterns of daily abundance for the two species may also explain findings from a previous study by Motley et al. (2017). In that study, *Leucopis* spp. from the Pacific Northwest were released in caged field experiments first in Tennessee in mid-May (at the southern edge of the eastern *A. tsugae* range) and then in upstate New York in early-June (at the northern edge of the eastern *A. tsugae* range) to evaluate survival and reproduction in eastern North America. Release-times at each site were staggered to synchronize with *A. tsugae* egg laying in the different regions. In New York, all F1 adults collected from enclosures were *L. argenticollis*, whereas those collected from the enclosures in Tennessee were a mix of *L. piniperda* and *L. argenticollis*. Given the findings of our study on the temporal presence of each species, a possible explanation for the findings of Motley et al. (2017) would be that species' composition was influenced by the temporally staggered collection of *Leucopis* spp. from the Pacific Northwest.

Our results also indicated differences in species proportions based on collection site. Although the effect of the site from which branches were collected did not correlate with *L. argenticollis* abundance, there was a relationship for *L. piniperda*. This is contrary to the findings of a previous study which found that site did not correlate with abundances of adult and immature specimens of either species (Rose et al. 2019). These differences may be explained, in part, by the large variance in our study, specifically in the Thurston Title Co. *L. piniperda* data set (Fig. 3b). Additionally, our sampling of only emerged adults versus all life stages may cause our study results to differ from those of previous studies (Rose et al. 2019). By excluding immature flies from our dataset, it was not possible to assess the effects of immature survival or interspecific competition by site, which is an area of consideration for further studies.

Our findings may have implications for the use of these *Leucopis* species as biological control agents of *A. tsugae* in eastern North America, especially considering the documented synchronicity issues for other Chamaemyiidae used for biological control (Smith and Coppel 1957, Mitchell and Wright 1967, Gaimari 1991). Our findings could imply that the two species work in tandem to impact *A. tsugae* populations and thus would both need to be released in eastern North America. Given the challenges of nondestructive differentiation of these two *Leucopis* species based on morphology (i.e., samples need to be DNA-barcoded), staggering specimen collections

in the West and subsequent releases in the East may increase the likelihood that both species of *Leucopis* spp. are released.

Broadly, our study of *L. argenticollis* and *L. piniperda* demonstrates the importance of fully understanding a species' niche in its native range to predict its implementation and impact as a biological control agent (Holmes 1973). Given that temporal investigations of these two species had only been conducted in aggregate, our study represents a novel investigation of *L. argenticollis* and *L. piniperda* resource partitioning patterns at a fine scale. Knowledge of these two species interspecific interactions, predator–prey relationships, and environmental requirements could improve the efficacy of their use as biological control agents of *A. tsugae*. With these insights, biological control programs can avoid the challenges of destructively sampling *Leucopis* spp. to identify them with DNA barcoding by staggering collection times in the West. This logistical modification may increase the likelihood that both species of *Leucopis* are released in the East with consequential improvements in their establishment and biological control efficacy.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Acknowledgments

We would like to thank Glenn Kohler, Sarah Grubin, and Xander Rose for their help identifying research sites. We thank Dr. Albert (Bud) Mayfield and Byran Mudder with the USDA Forest Service Southern Research Station for field support. We thank Liam Farley, Kerria Burns, Calla Sopko, Emily MacDonald, and David Owusu for laboratory assistance, Drs. Paul Schaberg (USDA Forest Service) and Scott Merrill (University of Vermont) for statistical advising and project design support. We thank reviewers' suggestions that overall strengthened the manuscript. This research was supported by funding from the USDA Forest Service, Hemlock Woolly Adelgid Initiative, and the Rubenstein School of Environment and Natural Resources.

References Cited

Aloo, T. C., and M. Karanja. 1986. The biological control of pine woolly aphid in Kenya using *Tetraphleps raoi* (Hemiptera: Anthocoridae). Edgerton Univ. College Res. Paper Ser. No. 10, Njoro, Kenya.

Angelini, C., A. H. Altieri, B. R. Silliman, and M. D. Bertness. 2011. Interactions among foundation species and their consequences for community organization, biodiversity, and conservation. BioScience. 61: 782–789.

Brantley, S., C. R. Ford, and J. M. Vose. 2013. Future species composition will affect forest water use after loss of eastern hemlock from southern Appalachian forests. Ecol. Appl. 23: 777–790.

Culliney T.W., J. W. Beardsley, Jr., and J. J. Drea 1988. Population regulation of the Eurasian pine adelgid (Homoptera: Adelgidae) in Hawaii. J. Econ. Entomol 81: 142–147.

deWaard, J. R., N. V. Ivanova, M. Hajibabaei, and P. D. Hebert. 2008. Assembling DNA barcodes. Analytical protocols. Methods Mol. Biol. 410: 275–293.

Ellison, A. M., M. S. Bank, B. D. Clinton, E. A. Colburn, K. Elliott, C. R. Ford, D. R. Foster, B. D. Kloeppel, J. D. Knoepp, G. M. Lovett, *et al.* 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Front. Ecol. Environ. 3: 479–486.

Gaimari, S. D. 1991. Use of silver flies (Diptera: Chamaemyiidae) for biological control of homopterous pests. Proc. Wash. St. Entomol. Soc. 53: 947–950.

Greathead, D. J. 1995. The *Leucopis* spp. (Diptera: Chamaemyiidae) introduced for biological control of *Pineus* sp. (Homoptera: Adelgidae) in Hawaii: implications for biological control of *Pineus boerneri* in Africa. Entomologist. 114: 83–90.

Grubin, S. M., D. W. Ross, and K. F. Wallin. 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.

Havill, N. P., S. D. Gaimari, and A. Caccone. 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. Control. 121: 23–29.

Havill, N. P., M. E. Montgomery, and M. Keena. 2011. Hemlock woolly adelgid and its hemlock hosts: a global perspective, chap. 1, pp. 3–14. *In* Onken B. and R. Reardon (eds.), Implementation status biological control of hemlock woolly adelgid FHTET-2011-04. US Dept. of Agric. Forest Service Forest Health and Technology Enterprise Team, Morgantown, WV.

Havill, N. P., L. C. Vieira, and S. M. Salom. 2014. Biology and control of Hemlock Woolly Adelgid. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV.

Havill, N. P., S. Shiyake, A. Lamb Galloway, R. G. Foottit, G. Yu, A. Paradis, J. Elkinton, M. E. Montgomery, M. Sano, and A. Caccone. 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.

Hebert, P. D., E. H. Penton, J. M. Burns, D. H. Janzen, and W. Hallwachs. 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.

Holmes, J. C. 1973. Site selection by parasitic helminths: interspecific interactions, site segregation, and their importance to the development of helminth communities. Can. J. Zool. 51: 333–347.

Humble, L. M. 1994. Recovery of additional exotic predators of balsam woolly adelgid, *Adelges piceae* (Ratzeburg) (Homoptera: Adelgidae), in British Columbia. Can. Entomol. 126: 1101–1103.

Jenkins, J. C., J. D. Aber, and C. D. Canham. 1999. Hemlock woolly adelgid impacts on community structure and N cycling rates in eastern hemlock forests. Can. J. For. Res. 29: 630–645.

Kizlinski, M. L., D. A. Orwig, R. C. Cobb, and D. R. Foster. 2002. Direct and indirect ecosystem consequences of an invasive pest on forests dominated by eastern hemlock. J. Biogeogr. 29: 1489–1503.

Kohler, G. R., K. F. Wallin, and D. W. Ross. 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.

Kohler, G. R., V. L. Stiefel, K. F. Wallin, and D. W. Ross. 2008. Predators associated with the hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. Environ. Entomol. 37: 494–504.

Kohler, G. R., V. L. Stiefel, K. Wallin, and D. W. Ross. 2007. Predators associated with the hemlock woolly adelgid (Hemiptera: Adelgidae) in the Pacific Northwest. University of Oregon Press.

Mausel, D. L., S. M. Salom, L. T. Kok, and J. G. Fidgen. 2008. Propagation, synchrony, and impact of introduced and native *Laricobius* spp. (Coleoptera: Derodontidae) on hemlock woolly adelgid in Virginia. Environ. Entomol. 37: 1498–1507.

Mausel, D. L., S. M. Salom, L. T. Kok, and G. A. Davis. 2010. Establishment of the hemlock woolly adelgid predator, *Laricobius nigrinus* (Coleoptera: Derodontidae), in the Eastern United States. Environ. Entomol. 39: 440–448.

McAlpine, J. F., and V. N. Tanasijtshuk. 1972. Identity of *Leucopis argenticollis* and description of a new species (Diptera: Chamaemyiidae). Can. Entomol. 104: 1865–1875.

McClure, M. S. 1987. Biology and control of hemlock woolly adelgid. Connecticut Agricultural Experiment Station, New Haven, CT.

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

Mitchell, R. G., and K. H. Wright. 1967. Foreign predator introductions for control of the balsam woolly aphid in the Pacific Northwest. J. Econ. Entomol. 60: 140–147.

Motley, K., N. P. Havill, A. L. Arsenault-Benoit, A. E. Mayfield, D. S. Ott, D. Ross, M. C. Whitmore, and K. F. Wallin. 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.

Orwig, D. A., D. R. Foster, and D. L. Mausel. 2002. Landscape patterns of hemlock decline in New England due to the introduced hemlock woolly adelgid. J. Biogeogr. 29: 1475–1487.

Pellmyr, O. 1989. The cost of mutualism: interactions between *Trollius europaeus* and its pollinating parasites. Oecologia. 78: 53–59.

Pompanon, F., E. Pettex, and L. Després. 2006. Patterns of resource exploitation in four coexisting globeflower fly species (*Chiastocheta* sp.). Acta Oecologica. 29: 233–240.

Quimby, J. W. 1996. Value and importance of hemlock ecosystems in the eastern United States, pp. 1–8. *In* S. M. Salom, T. C. Tigner, and R. C. Reardon (eds.), Proceedings of the first Hemlock Woolly Adelgid review, 12 October 1995, Charlottesville, VA. United States Department of Agriculture, Forest Service, For. Health Technol. Ent. Team, FHTET-96-10, Morgantown, WV.

R Core Team 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/

Rose A., Ross D., N. P. Havill, K. Motley, and K. F. Wallin. 2019. Relative abundance of three specialist predators of the hemlock woolly adelgid in the Pacific Northwest USA. Bull. Entomol. Res. 1–7.

Ross, R. M., R. M. Bennett, C. D. Snyder, J. A. Young, D. R. Smith, and D. P. Lemarie. 2003. Influence of eastern hemlock (*Tsuga canadensis* L.) on fish community structure and function in headwater streams of the Delaware River basin. Ecol. Freshw. Fish. 12: 60–65.

Schooley, H. O., J. W. E. Harris, and B. Pendrel. 1981. *Adelges piceae* (Ratz.), balsam woolly adelgid (Homoptera: Adelgidae), pp. 229–234. *In* J. S. Kelleher and M. A. Hulme(eds.), Biological control programmes against insects and weeds in Canada 1969–1980. Commonwealth Agricultural Bureaux, Farnham Royal, Slough, England.

Smith, B. C., and H. C. Coppel. 1957. Releases in North America and reviews of bionomics in Europe of insect predators of the balsam woolly aphid, *Adelges piceae* (Ratz.) (Homoptera: Adelgidae). Can. Entomol. 89: 410–420.

Stoetzel, M. B. 2002. History of the introduction of *Adelges tsugae* based on voucher specimens in the Smithsonian Institute National Collection of Insects. *In* Proc. Hemlock Woolly Adelgid East. U. S. Symp. Rutgers University, East Brunswick, NJ.

Vose, J. M., D. N. Wear, A. E. Mayfield, and C. Dana Nelson. 2013. Hemlock woolly adelgid in the southern Appalachians: control strategies, ecological impacts, and potential management responses. For. Ecol. Manag. 291: 209–219.

Walter, G. H. 1991. What is resource partitioning? J. Theor. Biol. 150: 137–143.

Ximenez-Embun, M. G., T. Zaviezo, and A. Grez. 2014. Seasonal, spatial and diel partitioning of *Acyrthosiphon pisum* (Hemiptera: Aphididae) predators and predation in alfalfa fields. Biol. Control. 69: 1–7.

Zilahi-Balogh, G. M. G., L. T. Kok, and S. M. Salom. 2002. Host specificity of *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), a potential biological control agent of the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). Biol. Control. 24: 192–198.

Zúñiga S. E. 1985. Eighty years of biological control in Chile. Historical review and evaluation of the projects undertaken (1903–1983). Agricultura Técnica 45: 175–183.