



Biological Control - Parasitoids and Predators

Hymenopteran Parasitoids of *Leucotaraxis argenticollis* (Diptera: Chamaemyiidae) and *Leucotaraxis piniperda*: Implications for Biological Control of Hemlock Woolly Adelgid (Hemiptera: Adelgidae)

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Abstract

The hemlock woolly adelgid (*Adelges tsugae* (Annand)) is a serious invasive pest of hemlock trees in eastern North America. Multiple biological control agents have been the focus of research aimed at pest management and conserving hemlock communities. Three promising *A. tsugae* specialist predators are the beetle *Laricobius nigrinus* (Fender) (Coleoptera: Derodontidae) and flies in the genus *Leucotaraxis* (Diptera: Chamaemyiidae), *Leucotaraxis argenticollis* (Zetterstedt), and *Leucotaraxis piniperda* (Malloch). However, these flies are vulnerable to parasitism by wasps in the genera *Pachyneuron* (Walker) (Hymenoptera: Pteromalidae) and *Melanips* (Walker) (Hymenoptera: Figitidae). This study explores parasitoid wasp interactions with these *Leucotaraxis* species in their native western North American range and potential impacts on the biological control program in the East. *Leucotaraxis*, *La. nigrinus*, and parasitoid emergences were observed from adelgid-infested foliage collected from Washington State and British Columbia in 2018, 2019, and 2020. Undescribed species of *Pachyneuron* and *Melanips* emerged from puparia as solitary parasitoids. Parasitoid emergence was positively correlated with *Leucotaraxis* emergence. Percent parasitism increased between February and July, with the months of June and July experiencing higher parasitoid emergence than *Leucotaraxis*. Differences in emergence patterns suggest that *Pachyneuron* may be more closely associated with *Le. argenticollis* as a host, and that *Melanips* may be associated with *Le. piniperda*. High parasitism in *Leucotaraxis* had no effect on *La. nigrinus* larval abundance, whereas the combined emergence of parasitoids and *Leucotaraxis* was positively correlated with *La. nigrinus*. This suggests that there is limited competition among these predators.

Key words: Adelgidae, biological control, parasitoid, host–parasitoid interactions

Hemlock woolly adelgid (*Adelges tsugae* (Annand), Hemiptera: Adelgidae) is an invasive forest pest of eastern hemlock (*Tsuga canadensis* (L.) Carrière, Pinales: Pinaceae) and Carolina hemlock (*Tsuga caroliniana* (Engelma)). *Adelges tsugae* was first discovered in the eastern United States in Richmond, Virginia in 1951 (Stoetzel 2002), and was likely introduced as early as the 1900s from southern Japan (Havill et al. 2006). Since then, it has spread to 21 states in

the eastern United States and two Canadian provinces (USDA Forest Service HWA Distribution map, Morgantown Field Office). Eastern hemlock is an ecologically important shade-tolerant foundation species, creating unique habitats throughout its range (Ellison et al. 2005). Unfortunately, it is highly susceptible to heavy infestation by *A. tsugae*, due to a lack of natural enemies and/or host tree resistance (McClure 1987, Havill et al. 2011). As a result, there has

been significant decline and mortality of hemlock in eastern North America (Orwig and Foster 1998, Eschtruth et al. 2006). Although systemic pesticide treatments are highly effective at managing *A. tsugae* on individual trees, landscape level implementation of chemical control is logistically and economically unsustainable (Cowles et al. 2006, Vose et al. 2013). An integrated management strategy utilizing chemical and biological methods shows promise for long-term landscape level control (Mayfield et al. 2020).

Adelges tsugae is not considered as a forest pest in the native Asian range (McClure et al. 1996) nor in western North America, where a native lineage feeds on western hemlock (*Tsuga heterophylla* (Raf.) Sargent) and mountain hemlock (*Tsuga mertensiana* (Bong.) Carrière) (Havill et al. 2016). In North America, *A. tsugae* has two parthenogenetic generations per year, an overwintering generation and a spring generation (McClure 1989). The spring generation consists of wingless progrediens and winged sexuparae that fly to spruce trees, where a sexually reproducing generation and gall formation occurs in the Asian range (Havill et al. 2016). The sexual generation fails to reproduce in North America because they lack a compatible spruce host species (McClure 1989, Havill et al. 2016). Parthenogenesis enables *A. tsugae* to become established through colonization by a single individual, leading to rapid dispersal and population growth (Tobin et al. 2013).

In its native Asian and western North American ranges, *A. tsugae* is predated by a suite of specialist and generalist predators (Yu et al. 2000, Kohler et al. 2008a, Shiyake et al. 2008). Research has been ongoing since the early 1990s to determine suitable candidates for use as biological control agents (Onken and Reardon 2011). Of these, the beetle *Laricobius nigrinus* (Fender) (Coleoptera: Derodontidae) has displayed successful establishment and dispersal in parts of the eastern United States (Mausel et al. 2010, Davis et al. 2012, Foley et al. 2019). Predation by *La. nigrinus* has been observed to significantly reduce the overwintering adelgid generation (Mausel et al. 2017, Jubb et al. 2020), but is negated by a population rebound with the spring generation (Crandall et al. 2020). These findings support the need for additional predators, especially specialists on the spring generation of *A. tsugae*.

Two predatory flies in the genus *Leucotaraxis* (Diptera: Chamaemyiidae), *Leucotaraxis argenticollis* (Zetterstedt), and *Leucotaraxis piniperda* (Malloch) (misidentified as *Leucopis atrifacies* (Aldrich) in Kohler et al. 2008a and 2008b; these both are members of a newly named genus, see Gaimari and Havill 2021), show promise as biological control agents. In the North American Pacific Northwest, these flies and *La. nigrinus* are the three most abundant predators and the only known specialists on *A. tsugae* (Kohler et al. 2008a, Kohler et al. 2016, Rose et al. 2019, Dietschler et al. 2021). *Leucotaraxis argenticollis* and *Le. piniperda* are also found in eastern North America; however, eastern lineages of both species are genetically distinct from their western relatives (Havill et al. 2018). The eastern biotypes of *Le. piniperda* and *Le. argenticollis* feed on adelgids in the genus *Pineus* (Shimer) (Hemiptera: Adelgidae) (Tanasijshtuk 2002, McAlpine and Tanasijshtuk 1972) and are very rarely found predating on *A. tsugae* (Havill et al. 2018; Gaimari and Havill 2021). The western biotypes of these *Leucotaraxis* species are promising as biocontrol agents due to their phenological synchrony and host specificity for *A. tsugae* (Grubin et al. 2011). Population peaks of these western *Leucotaraxis* larvae have been found to coincide with egg-laying by both generations of *A. tsugae* (Grubin et al. 2011, Rose et al. 2019).

One factor that may negatively influence the efficacy of these *Leucotaraxis* as biocontrol agents is parasitism. Flies in Chamaemyiidae are susceptible to several parasitoid species,

especially wasps in the genera *Melanips* (Walker) (Hymenoptera: Figitidae) and *Pachyneuron* (Walker) (Hymenoptera: Pteromalidae) (Ross et al. 2011). Both of these wasp genera have been reared from *Le. argenticollis* and *Le. piniperda* puparia with overall parasitism rates over 20% (Kohler et al. 2008b). Because of this, *Leucotaraxis* must be reared to adulthood before release to prevent their parasitoids from being introduced to novel environments. Globally, Noyes (2019) records the seven species of *Pachyneuron* associated with six genera of Chamaemyiidae (*Chamaemyia*, *Cremifania*, *Leucopina*, *Leucopis*, *Leucopomyia*, *Lipoleucopis*, and *Neoleucopis*; seven with the recent description of *Leucotaraxis*). Only two of the seven *Pachyneuron* species are known from the Nearctic region, whereas the remainder are Palearctic. The reported host records are often based on species catalogs, and many should be verified. In terms of parasitization of *Leucotaraxis*, *Pachyneuron* sp. is known from *Le. atrifacies* (Aldrich) (as *Leucopis*) (Herting 1978). Even less is known regarding host preference of *Melanips*. Specimens of undescribed *Melanips* with the remains of a *Leucopis* host are housed at The Natural History Museum, London, from both Kenya and India (M. L. Buffington, unpublished data). Little else is known about these parasitoids and their potential impact on *Leucotaraxis* as biological control agents.

This study investigated the parasitoid communities associated with heavy infestations of *A. tsugae* in the Pacific Northwest and the emergence rates of adult *Leucotaraxis* compared to *Pachyneuron* and *Melanips* parasitoids. From these emergence data, our objectives were to 1) examine the association between *Leucotaraxis* flies and the *Melanips* and *Pachyneuron* parasitoids emerging from the same foliage, 2) determine how parasitoid emergence numbers vary over time while rearing *Le. argenticollis* and *Le. piniperda* from collected foliage, 3) determine if the two genera of parasitoids are more closely associated with one of the two *Leucotaraxis* species, and 4) evaluate the implications of parasitism on competition between these *Leucotaraxis* and *La. nigrinus*.

Methods

Study Sites

Branches of *A. tsugae* infested western hemlock were collected in 2018, 2019, and 2020 to rear *Le. argenticollis*, *Le. piniperda*, and *La. nigrinus* for research and release for biological control in the introduced eastern range (Supp Table S1 [online only]). In 2018, the branches came from 10 field sites in Washington State and were sampled during three collection periods between February and May. In 2019, branches were collected from 16 field sites in Washington State from February to July, and from seven field sites in British Columbia, Canada, in May and June. In 2020, branches were collected from 15 field sites in Washington State from February to May, and eight field sites in British Columbia, Canada were sampled in February, March, and May. Temperature data were collected from the Lakshmi, Shannon Point, and Point Lawrence sites in 2019 and the Lakshmi, Shannon Point and Point Defiance sites in 2020 using a temperature data logger (Onset HOBO U23 Pro V2, Bourne, MA) placed on the north side of the bole of a collection tree at each site. Data were logged every 15 min.

The branches were shipped to the Sarkaria Arthropod Research Laboratory quarantine facility at Cornell University in Ithaca, NY (USDA APHIS permit P526P-18-00945). Branches were inserted into plastic tubs (Sterilite, 35.6 × 20.3 × 12.4 cm, Townsend, MA) with soaked floral foam (FloralCraft, Ludington, MI) to keep foliage hydrated. They were kept in custom acrylic cages (Leigh-Dale Specialties, Syracuse, NY) with 120-μ nylon mesh (Component

Supply Co., Sparta, TN) for ventilation and to prevent the mobile crawler stage of *A. tsugae* and other insects from escaping. Temperature data were recorded every 15 min in the quarantine facility in 2019 and 2020 (Onset HOBO U23 Pro V2, Bourne, MA), fluctuating between 12.2 and 19.7°C in 2019, and between 14.5 and 18.2°C in 2020. The greenhouse was kept under natural lighting conditions except when supplemental overhead lights were turned on during insect collections.

Specimen Collection and Identification

Insects were collected daily from cages between March 29 and June 29 in 2018, February 25 and August 16 in 2019, and February 20 and July 2 in 2020. At least 10 min prior to collection, overhead halogen lights were turned on in the greenhouse to attract arthropods upwards and out of the foliage. *Leucotaraxis* adults and *La. nigrinus* larvae were recorded daily while being removed from the cages for release as biocontrol agents. *Laricobius nigrinus* larvae were reared to the adult stage before removal from quarantine and release. *Leucotaraxis* adults from the sites with recorded temperature data were identified to species using the morphological and molecular methods in Dietschler et al. (2021). All parasitic Hymenoptera seen inside the cages were aspirated into clear 9-dram vials. Specimens were immediately frozen for at least 3 d before being sorted into individual vials. Specimens that emerged in lower numbers and were not thought to be parasitoids of *Leucotaraxis* were identified to family using the keys in Goulet and Huber (1993). Parasitoids of high abundance were identified to genus as *Pachyneuron* using Bouček and Heydon (1997), and to *Melanips* using Buffington et al. (2020). Throughout this paper, ‘parasitoids’ refers specifically to these two genera, whereas other collected Hymenoptera will be referred to as ‘non-focal parasitoids’.

Several *Pachyneuron* and *Melanips* specimens were sent to the USDA-ARS Systematic Entomology Laboratory for further identification. Ethanol-preserved specimens were dehydrated through increasing concentrations of ethanol and transferred to hexamethyldisilazane (HMDS) (Heraty and Hawks 1998) before point-mounting. Parasitoid specimens were identified using a Leica M205C stereomicroscope with 10X oculars and a Leica LED ring light source for point-mounted specimen observation.

In 2020, a subset of branches from several sites was dissected to separate a total of 137 *Leucotaraxis* puparia into individual 2-oz deli cups. These cups were kept in an incubator at 16°C and checked daily until a parasitoid or *Leucotaraxis* adult emerged. Individual parasitoids and the puparium they eclosed from were placed in vials with 90% ethanol, and then identified to genus. This was done to confirm that the *Pachyneuron* and *Melanips* being collected were emerging from *Leucotaraxis* puparia (Kohler et al. 2008b), to monitor for any other parasitoid species emerging from the puparia, and to determine if these parasitoids were solitary or gregarious.

Statistical Analysis

Site, collection period, and date of emergence were recorded for all specimens. We performed a Pearson’s correlation to evaluate the relationship between parasitoid and *Leucotaraxis* emergence. Parasitoid and *Leucotaraxis* counts were natural log transformed using $(\ln(x + 1))$ to reduce skew in the data. We also performed a Pearson’s correlation to determine if there is a density dependent relationship between percent parasitism and the population of immature *Leucotaraxis*. Percent parasitism (P) was calculated for each site-year by dividing the number of parasitoids (p) that emerged by the total number of parasitoids and *Leucotaraxis* (l) that emerged

and multiplying by 100, $P = (p/(p + l) * 100)$. This was done with the assumption that one parasitoid emerges from an individual puparium, based on observations of solitary parasitoid emergence from *Leucotaraxis* puparia in deli cups. We do not know exactly how many *Leucotaraxis* puparia were present or how many were parasitized; we only recorded the number of adult flies and parasitoids that emerged. As a result, any unclosed puparia were not included in analyses using percent parasitism. Immature *Leucotaraxis* populations were estimated by adding together adult *Leucotaraxis* and parasitoid emergence. Percent parasitism and immature *Leucotaraxis* counts were both natural log transformed using $(\ln(x + 1))$ to reduce skew in the data.

To examine temporal variation in parasitoid emergence from *A. tsugae* infested foliage, we performed a one-way ANOVA comparing percent parasitism from rearing material collected in different months. Percent parasitism (P) was calculated for each site per collection period. A post-hoc Tukey HSD test was used to determine significant differences between months.

The accumulated number of heating degree days was calculated using the modified sine wave method (Allen 1976) for the Lakshmi, Shannon Point, Point Lawrence, and Point Defiance sites in 2019 and 2020 using the methods from Dietschler et al. (2021). As in Dietschler et al. (2021), a base temperature of 4°C was used based on developmental thresholds found for *A. tsugae* progrediens (Salom et al. 2002, Tobin and Turcotte, 2018). Due to a lack of data on temperature thresholds for *Leucotaraxis* and their parasitoids, 4°C was used because of the association between *A. tsugae* and *Leucotaraxis*. This allows for standardized emergence timing across collection periods. Differences in emergence distribution between each genus of parasitoid and each *Leucotaraxis* species were examined using a non-parametric two-sample Kolmogorov–Smirnov test for each site (Conover 1971). Graphs comparing *Melanips*, *Pachyneuron*, *Le. argenticollis*, and *Le. piniperda* emergence in relation to heating degree day accumulation were used to visualize patterns suggesting host associations.

To examine the effects of parasitism on competition between *Leucotaraxis* and *La. nigrinus*, we performed a Pearson’s correlation comparing *La. nigrinus* larval abundance to percent parasitism at sites where both predator genera were found. To look at coexistence between *Leucotaraxis* and *La. nigrinus* larvae, we performed a Pearson’s correlation comparing *La. nigrinus* larval abundance and the sum of *Leucotaraxis* and parasitoid emergence. The combination of adult *Leucotaraxis* and parasitoid emergence serves as an estimate for the number of *Leucotaraxis* larva that fed and developed on *A. tsugae* in potential co-occurrence with *La. nigrinus* larvae. *Leucotaraxis* and *La. nigrinus* data were natural log transformed using $(\ln(x + 1))$ to reduce skew. Data analysis was performed using R Studio version 4.0.3 (R Core Team 2020). Graphs and plots were made in R Studio using the ggplot2 package (Wickham 2016).

Results

Parasitoid Identification

Overall, 19,285 *Leucotaraxis* adults and 15,844 *La. nigrinus* larvae were collected. We collected 6,411 parasitic Hymenoptera, of which 1,548 were *Pachyneuron*, 3,044 were *Melanips* (Table 1), and 1,819 were non-focal parasitoids from 19 families (Supp Table S2 [online only]). The species of *Pachyneuron* and *Melanips* found are undescribed, and, due to the taxonomic difficulty of both genera, were not assigned a species concept.

Evidence for *Leucotaraxis* Parasitism

Of the 137 *Leucotaraxis* puparia that were observed, fifteen had parasitoids emerge from them. *Pachyneuron* emerged from four puparia, and *Melanips* emerged from eleven. All parasitoids emerged as a single parasitoid from an individual puparium.

There was a significant, positive correlation between parasitoid emergence and *Leucotaraxis* emergence ($r_{(54)} = 0.672$, P -value < 0.001 ; Fig. 1). There was no significant correlation between percent parasitism and immature *Leucotaraxis* abundance ($r_{(52)} = 0.49$, P -value = 0.096). Of 56 total sites, 42 had both *Leucotaraxis* and parasitoids present, and two sites had no emergence of flies or parasitoids. Sites that had parasitoid emergence with no *Leucotaraxis* emergence, or vice versa, only had low levels of emergence (≤ 18 parasitoids, ≤ 38 *Leucotaraxis*). This may be due to sampling bias introduced by the timing of collections (see Fig. 2) or limited sampling at these sites.

Effect of Collection Period on *Leucotaraxis* Parasitism

The month that foliage was collected had a significant effect on percent parasitism ($F_{(5,111)} = 13.65$, P -value < 0.001 ; Fig. 2). The percent parasitism observed in April was significantly lower than in June (P -value = 0.001) and July (P -value = 0.041), but was not significantly lower than May (P -value = 0.412). There was no significant difference between the months of February, March, and April, and no significant difference between the months of May, June, and July.

Parasitoid Emergence and Associations with *Leucotaraxis piniperda* and *Leucotaraxis argenticollis*

Emergence timing, expressed as accumulated heat degree days using a base temperature of 4°C, differed significantly between *Pachyneuron* and *Melanips* at three of the six site-years. The two parasitoid genera had separate emergences in 2019 at Lakshmi ($D = 0.402$, P -value = 0.003) and Shannon Point ($D = 0.396$, P -value = 0.003), and in 2020 at Point Defiance ($D = 0.389$, P -value < 0.001). Emergence distributions were not different in 2019 at Point Lawrence ($D = 0.375$, P -value = 0.441) or in 2020 at Lakshmi ($D = 1$, P -value = 1) and Shannon Point ($D = 0.4$, P -value = 0.854), potentially due to the smaller sample size at these sites. As shown in Dietschler et al. (2021) and in this paper (Fig. 3), there were differences in the timing of emergence between the two *Leucotaraxis* host species. *Leucotaraxis piniperda* has a single peak in emergence, with peaks in emergence for *Le. argenticollis* occurring before and after. This paper and Dietschler et al. (2021) use the same *Leucotaraxis* emergence data, whereas parasitoid data are unique to this paper.

Differences in emergence distributions between the flies and parasitoids were found. Many of the emergence patterns in Fig. 3, especially Point Defiance in 2020, show a time lag between *Leucotaraxis* emergence and subsequent parasitoid emergence. *Pachyneuron* emergence distributions were different from *Le. argenticollis* in 2019 at Lakshmi ($D = 0.921$, P -value < 0.001), Shannon Point ($D = 0.433$, P -value < 0.001), and Point Lawrence ($D = 0.929$, P -value < 0.001) and in 2020 at Shannon Point ($D = 1$, P -value = 0.006) and Point Defiance ($D = 0.887$, P -value < 0.001). *Pachyneuron* emerged shortly after an initial wave of *Le. argenticollis* emergence (Fig. 3). *Pachyneuron* emergence time was significantly different from *Le. piniperda* in 2019 at Lakshmi ($D = 0.477$, P -value < 0.001) and Point Defiance in 2020 ($D = 0.859$, P -value < 0.001). There is considerable overlap in emergence between *Pachyneuron* and *Le. piniperda*, though *Pachyneuron* seems to continue emerging after *Le. piniperda* emergence drops.

Table 1. Total number of *Leucotaraxis*, *Laricobius nigrinus*, *Melanips*, *Pachyneuron*, and non-focal parasitoids collected

Species	2018	2019	2020	Total
<i>Leucotaraxis</i>	2,262	10,291	6,732	19,285
<i>Laricobius nigrinus</i>	1,345	4,837	9,662	15,844
<i>Melanips</i>	1,007	1,358	679	3,044
<i>Pachyneuron</i>	270	940	338	1,548
Non-focal parasitoids	228	816	775	1,819

Parasitoid vs *Leucotaraxis* Emergence

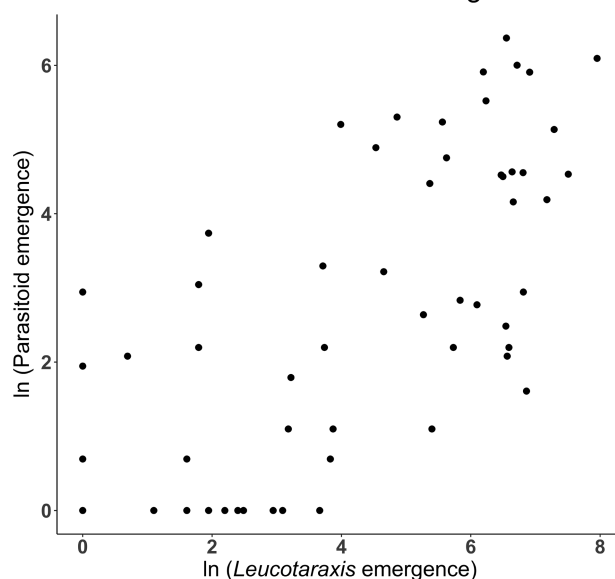


Fig. 1. Pearson's correlation of parasitoid emergence and total *Leucotaraxis* emergence for all sites and years ($r_{(54)} = 0.672$, P -value < 0.001 , $\alpha = 0.05$). Data have been transformed on both axes using the natural log ($\ln(x + 1)$). 'Parasitoid emergence' refers to the combined emergence of *Melanips* and *Pachyneuron*.

Effect of collection time on *Leucotaraxis* parasitism

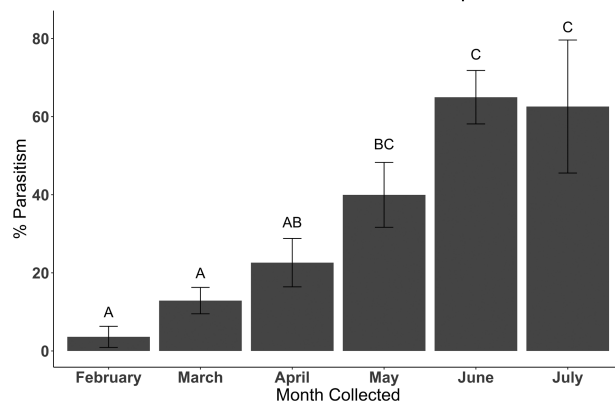


Fig. 2. One-way ANOVA showing differences in percent parasitism between collection months ($F_{(5,111)} = 13.65$, P -value < 0.001). Letters denote significant differences ($\alpha = 0.05$) based on Tukey's HSD test.

Pachyneuron emergence time was not different from *Le. piniperda* in 2019 at Shannon Point ($D = 0.453$, P -value = 0.115) and Point Lawrence ($D = 0.5$, P -value = 0.051) and in 2020 at Lakshmi (D

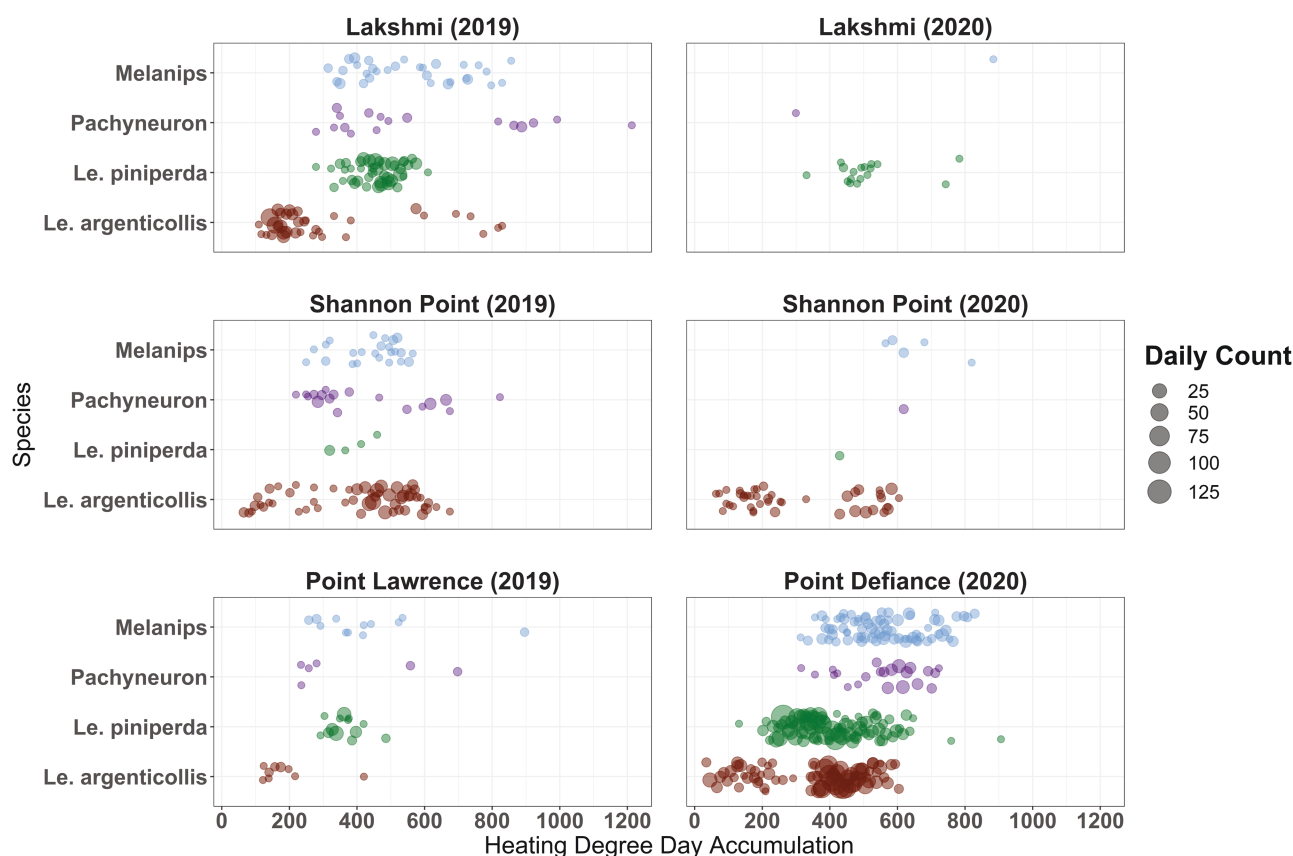


Fig. 3. Daily emergence plotted against the amount of heat degree days (base temperature 4°C) accumulated for *Leucotaraxis argenticollis* and *Leucotaraxis piniperda*, and wasps *Melanips*, and *Pachyneuron* for each site in 2019 and 2020.

= 1, P -value = 0.2997) and at Shannon Point ($D = 1$, P -value = 0.181).

Melanips emergence distributions were distinct from *Le. piniperda* in 2019 at Lakshmi ($D = 0.425$, P -value < 0.001), Shannon Point ($D = 0.647$, P -value = 0.009), and Point Lawrence ($D = 0.403$, P -value = 0.025), and in 2020 at Point Defiance ($D = 0.685$, P -value < 0.001), but there was no difference at Shannon Point ($D = 1$, P -value = 0.071) or at Lakshmi ($D = 1$, P -value = 0.2997) in 2020. *Melanips* emergence began later than *Le. argenticollis* in 2019 at Lakshmi ($D = 0.938$, P -value < 0.001), Point Lawrence ($D = 0.929$, P -value < 0.001), and in 2020 at Shannon Point ($D = 0.891$, P -value < 0.001) and Point Defiance ($D = 0.672$, P -value < 0.001). In 2019, the *Melanips* emergence distribution was not different from *Le. argenticollis* at Shannon Point ($D = 0.137$, P -value = 0.618).

Indirect Effects of *Leucotaraxis* Parasitoids on *A. tsugae* Predator *Laricobius nigrinus*

There was no correlation between percent parasitism on *Leucotaraxis* and the number of *La. nigrinus* larvae collected ($r_{(41)} = -0.089$, P -value = 0.570). When both predator genera are present, there is a positive correlation between *La. nigrinus* larval abundance and the sum of *Leucotaraxis* and parasitoid emergence ($r_{(41)} = 0.585$, P -value < 0.001; Fig. 4).

Discussion

Parasitoid Identification

Pachyneuron (Walker) consists of over 50 species globally with 12 known from the Nearctic and 28 from the Palearctic. Some regional

species treatments exist: Szelenyi (1942; Palearctic), Sureshan (2003; India), Graham (1969; Europe), Gibson (2001; Australia), Narendran et al. (2007; Middle East), and Kamijo and Takada (1973; Japan). In other regions, the species are unrevised. Most species are hyperparasites of Aphididae or other Hemiptera (Coccoidea, Psylloidea) through their Braconidae (Ichneumonidae) or Aphelinidae and Encyrtidae (Chalcidoidea) primary parasitoids, or are primary parasitoids or hyperparasitoids of the predators of these plant herbivores (Gibson 2001, Noyes 2019). *Melanips* (Walker) consists of 31 species globally (Buffington et al. 2020) and is a taxonomically problematic genus within Figitidae. Morphologically these species appear to be cynipids, but their biology and phylogeny put them securely in Aspicerinae (Figitidae) (Buffington et al. 2012). The group is currently being evaluated as its own subfamily (Mata-Casanova et al., submitted). Adding to the taxonomic difficulty of *Melanips* is the lack of any revision of the genus. Together, *Pachyneuron* and *Melanips*, though unrelated, share the commonality that species currently assigned to these genera are not well defined; hence, species of these genera encountered in this current study cannot be readily assigned to a species concept.

Evidence for *Leucotaraxis* Parasitism

Observation of individually reared puparia shows that *Pachyneuron* and *Melanips* are the dominant parasitoid genera that use these *Leucotaraxis* species as hosts. All observed puparia containing parasitoids had a single wasp emerge, indicating that parasitoids in both genera are solitary rather than gregarious. This is consistent with other studies that have observed parasitoids of these *Leucotaraxis* species (Grubin et al. 2011, Kohler et al. 2008b). Across

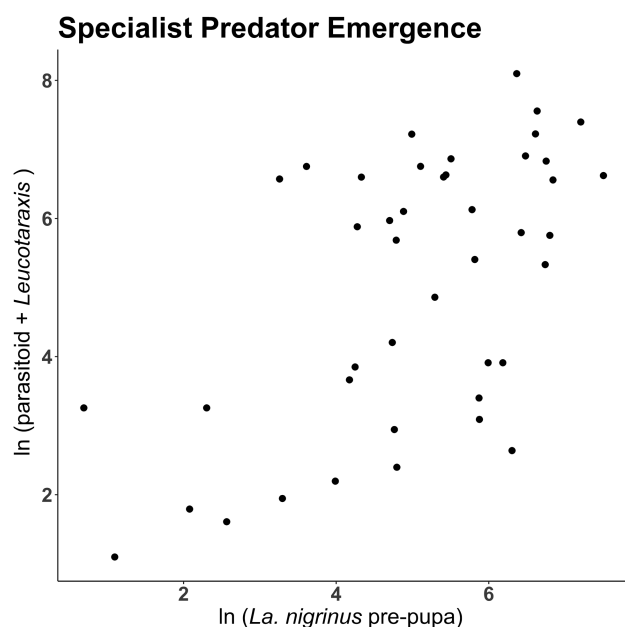


Fig. 4. Pearson's correlation of *Laricobius nigrinus* larval abundance and the sum of *Leucotaraxis* and parasitoid emergence at each site ($r_{(41)} = 0.585$, P -value < 0.001). Data have been transformed on both axes using the natural log ($\ln(x + 1)$).

all site-years, the emergence totals of *Pachyneuron* and *Melanips* increased with *Leucotaraxis* emergence totals, suggesting an association between these parasitoids and *Leucotaraxis* (Fig. 1). However, there was no significant correlation between percent parasitism and immature *Leucotaraxis* abundance, suggesting that parasitism rate is not density dependent. More work should be done investigating these host–parasitoid interactions to further understand how these parasitoids influence *Leucotaraxis* populations, and consequentially *A. tsugae* biological control.

Non-focal parasitoid families had relatively low emergence compared to *Pachyneuron* and *Melanips*, indicating that these are likely not important parasitoids of *Leucotaraxis* or are parasitoids of other insects associated with hemlock. With both focal parasitoids emerging in high numbers from the same foliage as *Leucotaraxis*, this highlights the importance of careful cleansing of wild-collected *Leucotaraxis* and releasing only adult flies for biological control. It also provides an incentive to establish clean laboratory colonies in the East for research purposes.

Effect of Collection Period on *Leucotaraxis* Parasitism

When comparing *Leucotaraxis* rearing cages, foliage collected in the later months had increased percent parasitism compared to foliage collected earlier in the year (Fig. 2). Parasitoids begin to increase between April and July and exceed *Leucotaraxis* emergence in June and July. These patterns suggest that collecting *Leucotaraxis* immatures to rear for biological control earlier in the year could help reduce the number of parasitoids in the sampled material.

Several sites showed a time lag between adult *Leucotaraxis* emergence and parasitoid emergence, with significant overlap at other sites (Fig. 3). As a result, it may be best to collect foliage when *Leucotaraxis* larvae are active, but before parasitoid emergence. Previous studies have shown that *Leucotaraxis* larva population levels peak in synchrony with the egg laying stage of both *A. tsugae*

generations (Grubin et al. 2011, Kohler et al. 2016). In the Pacific Northwest, *A. tsugae* eggs are present February to mid-May, and again from June to mid-July (Zilahi-Balogh et al. 2003). Due to the increase in parasitoid emergence seen later in the year (Fig. 2), we suggest that collecting Pacific Northwestern foliage for *Leucotaraxis* rearing during the egg stage of the *A. tsugae* spring generation may reduce the parasitism rate in the collected sample.

Performing *Leucotaraxis* collections during times of the year with lower parasitoid emergence may reduce the number *Leucotaraxis* lost to parasitism and reduce time needed to cleanse collections of parasitoids. However, with adult *Leucotaraxis* emerging during both generations of *A. tsugae* (Dietschler et al. 2021), collections will need to be continued through June in order to pair releases with the appropriate *A. tsugae* phenology, when *A. tsugae* eggs are abundant in the introduced range in eastern North America (McClure 1987, Gray and Salom 1996, Mausel et al. 2008, Joseph et al. 2011). Restricting collections to a specific time period may also favor the collection of one *Leucotaraxis* species over the other due to their alternating emergence patterns (Neidermeier et al. 2020, Dietschler et al. 2021). Future research on what stage of *Leucotaraxis* development is initially parasitized by these wasps may further inform timing of foliage collection.

Parasitoid Emergence and Associations with *Leucotaraxis piniperda* and *Leucotaraxis argenticollis*

Emergence distributions for *Pachyneuron* and *Melanips* were significantly different from each other, demonstrating variation in phenology and host association. Separate emergence distributions were also shown for *Le. argenticollis* and *Le. piniperda*. Parasitoid emergence was independent from host emergence, with parasitoid emergence occurring slightly after adult *Leucotaraxis* (Fig. 3). Parasitoid emergence likely coincides with the presence of immature hosts at the preferred developmental stage for oviposition, after the adult flies have mated and reproduced.

Previous studies have found that *Le. argenticollis* and *Le. piniperda* have non-overlapping adult emergence peaks within a year (Neidermeier et al. 2020) that are consistent and predictable (Dietschler et al. 2021). *Leucotaraxis argenticollis* has a bimodal emergence, emerging before and after *Le. piniperda* (Dietschler et al. 2021). In addition, we also observed some evidence for a bimodal emergence pattern in *Pachyneuron*, especially at Lakshmi, Shannon Point, and Point Lawrence in 2019 (Fig. 3). Lab-reared specimens of the species *Pachyneuron altiscutum* (Howard), a parasitoid of the chamaemyiid *Neoleucopis obscura* (Haliday), have previously shown evidence of having two generations a year (Brown and Clark 1957). Meanwhile, *Le. piniperda* and *Melanips* seem to have single peaks in emergence. These emergence trends provide evidence that *Pachyneuron* may use *Le. argenticollis* as a host, and that *Melanips* may use *Le. piniperda*. Further research is needed to determine if these are strict associations or if these parasitoids can successfully complete development on both *Leucotaraxis* species.

Indirect Effects of *Leucotaraxis* Parasitoids on *A. tsugae* Predator *Laricobius nigrinus*

Understanding the direct and indirect effects of parasitism on predator community dynamics is important to successful implementation of management strategies utilizing a multi-predator approach. Parasitism pressure on these *Leucotaraxis* could potentially change competitive interactions with *La. nigrinus*, either by directly influencing *Leucotaraxis* population numbers or via non

consumptive effects (Abram et al. 2019). Our results indicate that heavy parasitism of these *Leucotaraxis* has no effect on *La. nigrinus* larval abundance, at least when prey densities are high, as in our collections. This may be an indicator that there is limited competition between *Leucotaraxis* and *La. nigrinus*, possibly due to temporal (Rose et al. 2019, Neidermeier et al. 2020, Dietschler et al. 2021) or spatial niche partitioning. Larval *La. nigrinus* abundance is positively correlated with the combined emergence of *Leucotaraxis* adults and the parasitoids (Fig. 4). Since both parasitoids are solitary, combining parasitoid and *Leucotaraxis* emergence gives an estimate of how many *Leucotaraxis* larva were at a site, which is the life stage that could compete most directly with *La. nigrinus* larva for resources. These findings suggest that there is limited competition between these *Leucotaraxis* and *La. nigrinus*, indicating that they may be compatible for use as biological control agents simultaneously. This could be important for effective *A. tsugae* biological control, as the co-existence of both biological control agents would allow for predation on all adelgid generations. Further research should be done to look at interactions between these predators when *A. tsugae* populations are low, which would likely increase competition over a shared resource.

Due to the constraints of working with these parasitoids in a quarantine facility in the eastern United States, this research was based on emergence data gathered from specimens that were immediately killed. Experiments to increase our understanding of the behavioral ecology of *Pachyneuron* and *Melanips* parasitoids of these *Leucotaraxis* and their impacts on predators are important to increasing biological control agent collection efficiency and implementation. This includes experiments to verify host associations and specialization, effects of parasitism on *Leucotaraxis* behavior and development prior to host death, and the host stage initially parasitized. We have evidence that *Melanips* initially parasitizes the larval stage of *Leucotaraxis* and emerges after it pupariates. Eight *Leucotaraxis* that were collected as larvae and reared on *A. tsugae* had *Melanips* emerge from them in the puparial stage (N. P. Havill, unpublished data). It is not known whether *Melanips* can parasitize both the larvae and puparia, and the stage(s) in which *Pachyneuron* oviposits is(are) still unknown. If parasitism begins at the egg stage or larval instars when *Leucotaraxis* are still actively feeding, this may have consequences for predation efficiency. Some parasitoids have been shown to modify host behavior, either by directly altering host physiology and chemistry from within a host or through non-consumptive effects (Slansky 1986, Abram et al. 2019). Possible changes include altered foraging behavior, increased or decreased food consumption, and host development time (Guillot and Vinson 1973, Senthamizhselvan and Muthukrishnan 1989). Similar changes in behavior in these *Leucotaraxis* species could have direct impacts in their efficiency at controlling *A. tsugae* and interspecies competition with other predators. Potential changes in host behavior or physiology could also change competition dynamics between parasitized and unparasitized conspecifics (Sisterson and Averill 2003, Cameron et al. 2005). The presence or absence of native eastern parasitoids of *Leucotaraxis* in the introduced range may have consequences for arthropod community dynamics, and will be important to understand because of their potential impacts on *Leucotaraxis* establishment and *A. tsugae* control.

Related fly species in the United States experience heavy parasitism by wasps in the genera *Pachyneuron* and *Melanips*. Chamaemyiid flies that were studied for biological control of balsam woolly adelgid, *Adelges piceae*, have had recorded parasitism by the wasp *Pachyneuron altiscutum* (Brown and Clark 1956). *Neoleucopis obscura* in particular had parasitism rates of 20 percent or more in eastern Canada (Brown and Clark 1957). *Leucopis bellula*

(Williston), a natural enemy of coccid scales on prickly pear cactus, had recorded parasitism by *Pachyneuron mucronatum* (Girault), and *Melanips lomevya* (Pujade-Villar & Vanegas-Rico) (Gilreath and Smith 1988, Mendel et al. 2020). *Pachyneuron* and *Melanips* have also been collected from chamaemyiid puparia in co-occurrence with *Le. piniperda* in the eastern United States, though it is unknown if these are the same *Pachyneuron* species as in the west (N. P. Havill, unpublished data). As western biotypes of *Le. piniperda* and *Le. argenticollis* become established in eastern North America, it will be important to monitor their populations for parasitism should parasitoids of native chamaemyiid flies, especially parasitoids of eastern biotypes of *Le. argenticollis* and *Le. piniperda*, switch to the introduced western biotypes. More research is needed on how these parasitoids could impact the efficacy of *Leucotaraxis* as a biological control agent, and how this would influence the wider community of *A. tsugae* predators.

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Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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