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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

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Abstract

Adelges tsugae infested western hemlock trees were sampled periodically for 1 year at two locations in Oregon and Washington to compare the phenology and abundance of three associated predators (*Leucopis argenticollis*, *Leucopis piniperda*, and *Laricobius nigrinus*) and their host. On each sample date, two 3–10 cm long terminal twigs were collected from each tree and brought to the laboratory to count all life stages of *A. tsugae* and the three predators. Peak larval abundance of *Leucopis* spp. and *La. nigrinus* coincided with the presence of *A. tsugae* adults and eggs. *Leucopis* spp. larvae were present for a much longer period of time than were *La. nigrinus* larvae. Furthermore, *Leucopis* spp. larvae were present during both the progrediens and sistens egg stages, while *La. nigrinus* larvae were only present during the progrediens egg stage. Overall, we collected 2.3–3.5 times more *Leucopis* spp. of all life stages than *La. nigrinus*. These results support the continued study of *Leucopis* spp. from the Pacific Northwest as biological control agents for *A. tsugae* in the Eastern USA.

Keywords: hemlock woolly adelgid, silver flies, biological control

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Introduction

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is an introduced insect that feeds on eastern hemlock (*Tsuga canadensis* (L.) Carrière) and Carolina hemlock (*Tsuga caroliniana* Engelmann) in the Eastern USA. *Adelges tsugae* was first reported in the Eastern USA near Richmond, Virginia, in 1951 (Stoetzel, 2002) and was subsequently determined to have been introduced there from Southern Japan (Havill *et al.*, 2006). In the 1980s, *A. tsugae* began spreading rapidly throughout the range of eastern hemlock causing high levels of tree mortality. It is now found in eighteen eastern states from Georgia to Southern Maine (Havill *et al.*, 2011).

Adelges tsugae is a minute (0.4–1.4 mm long), sucking insect that remains stationary for most of its life within cottony flocculence near the base of hemlock needles. *Adelges tsugae* inserts its mouthparts into the stem and feeds on the xylem ray parenchyma. There are two generations per year, with oviposition occurring in late winter (progrediens eggs) and early summer (sistens eggs). A mobile crawler emerges from the egg and seeks out an unoccupied leaf base where it settles and molts to a first instar nymph (McClure, 1987). In early spring and

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summer, the crawler stage can disperse to new trees via wind, the nursery trade, birds, mammals, or humans (McClure, 1990; Ward *et al*., 2004). The first instar nymph of the sistens generation will undergo a 2 to 4 months aestival diapause during late summer. There is no reported sexual reproduction of *A. tsugae* in North America; all individuals reproduce parthenogenetically.

Since the early 1990s there has been considerable effort devoted to the development of classical biological controls for *A. tsugae*. Because *A. tsugae* has no reported parasitoids, the most promising biological control agents are adelgid specialist predators and/or entomopathogens (Cheah *et al*., 2004). Explorations in its native ranges in Asia and western North America have identified several potential biological control agents (Onken & Reardon, 2011). To date, the biological control program has focused primarily on two predatory beetles, *Sasajiscymnus tsugae* (Sasaji and McClure), a coccinelid imported from Japan, and *Laricobius nigrinus* Fender, a derodontid imported from western North America. Between 1995 and 2010, over 2 million *S. tsugae* were released at more than 400 sites in 16 eastern states (Cheah, 2011). Between 2003 and 2010, several hundred thousand *La. nigrinus* adults and eggs were released at hundreds of sites in 14 eastern states (Mausel *et al*., 2011). Despite the large numbers of *S. tsugae* and *La. nigrinus* released and their establishment at numerous sites, there is no indication that they are significantly impacting *A. tsugae* populations or reducing the rate of hemlock mortality. Consequently, exploration for additional predators to release in the Eastern USA is continuing.

A beat sampling survey of 116 *A. tsugae* infested western hemlocks (*Tsuga heterophylla* (Raf.) Sarg.) across 16 sites in Western Oregon and Washington over 23 months resulted in the collection of over 6000 adult and immature predators representing 55 species from 43 genera, 14 families, and four orders (Kohler *et al*., 2008). *Laricobius nigrinus* was found to be the most abundant, comprising 43% of all predators collected. Collectively, two species of *Leucopis* (Diptera: Chamaemyiidae), *Le. argenticollis* Zetterstedt and *Le. piniperda* Malloch (misidentified as *Le. atrifacies* Aldrich, see Grubin *et al*., 2011), were the second most abundant predators, comprising 16% of the total. Since we do not know the efficacy of beat sampling for the different species, the actual relative abundance of *La. nigrinus* to *Leucopis* species is likely different than these numbers suggest. The ratio of immatures to adults was over three times higher for the chamaemyiids (9.2) compared with the derodontids (2.6) or hemerobiids (3.1), the third most abundant group, suggesting that beat sampling was less effective at collecting adult chamaemyiids than adults of other species, and that they are more abundant relative to the other predators than indicated by counts from beat sampling. *Laricobius nigrinus*, *Le. argenticollis*, and *Le. piniperda* were the only adelgid specific predators that were both frequently encountered and abundant in the survey. This was the first record of either *Le. argenticollis* or *Le. piniperda* collected in association with *A. tsugae*, although both species have been collected in association with other *Pineus* and *Adelges* species in other parts of North America (Ross *et al*., 2011). The abundance of *Leucopis* spp. larvae and *Le. argenticollis* adults was positively correlated with *A. tsugae* population and no *Leucopis* spp. were collected from uninfested *T. heterophylla* during the survey. The results of this survey suggest that the two *Leucopis* spp. merit further study as potential biological control agents for use in the Eastern USA.

The primary objective of the study reported here was to evaluate the degree of synchrony between life cycles of the two *Leucopis* spp. pooled and *A. tsugae* to further evaluate their potential as biological control agents. Specifically, we were interested in determining what life stage of *A. tsugae* was present when actively feeding *Leucopis* spp. larvae were most abundant. In addition, we recorded the abundance of *La. nigrinus* on the same trees for comparison with *Leucopis* spp.

Materials and methods

Heavily infested *T. heterophylla* that would support populations of chamaemyiid predators are most likely to be found in orchard or ornamental plantings. Six *T. heterophylla* infested with *A. tsugae* were sampled at 1–4 week intervals at a seed orchard near St. Paul, Oregon from May 2007 to May 2008. Seven *T. heterophylla* infested with *A. tsugae* were sampled at 2–3 week intervals in an ornamental planting in Olympia, Washington from September 2007 to August 2008. On each collection date, two terminal twig samples per tree were cut from randomly selected *A. tsugae* infested branches below 2.5 m in height. The length of twig samples ranged from 3 to 10 cm. Twig samples were brought to the laboratory and carefully inspected with a dissecting microscope. All stages of living *A. tsugae* were counted, except eggs, which were always present with adults. The instar of *A. tsugae* nymphs was determined by counting exuvia (Zilahi-Balogh *et al*., 2003). Counts of third and fourth instar *A. tsugae* nymphs were pooled because they were sometimes difficult to distinguish. All live *Leucopis* spp. eggs, larvae, and puparia were counted. *Leucopis* spp. have three larval instars (McAlpine & Tanasijtshuk, 1972; Tanasijtshuk, 2002). The first instar is identifiable by size and lack of pigment. Counts of second and third instar *Leucopis* spp. larvae were pooled because they are difficult to distinguish reliably. Larvae and adults of *La. nigrinus* were also counted. The density of insects is reported as individuals per cm twig length.

Analyses

Mean densities were calculated separately for each location at each sample date. Means were calculated for all *Leucopis* spp. larval instars pooled, *La. nigrinus* larvae, *A. tsugae* instars 2–4 pooled, and *A. tsugae* adults and eggs. *Leucopis* spp. and *La. nigrinus* larval densities were graphed against *A. tsugae* instars 2–4 and *A. tsugae* adults and eggs.

Results

Leucopis spp. larvae can be found throughout the year on *A. tsugae* infested twigs in the Pacific Northwest (PNW) (figs 1 and 2). All *Leucopis* spp. eggs were found at the base of hemlock needles in contact with *A. tsugae* wool. All *Leucopis* spp. and *La. nigrinus* larvae were found within *A. tsugae* wool, either in contact with live *A. tsugae* or in unoccupied ovisacs.

The approximate period when *A. tsugae* progrediens and sistens eggs were observed across the two locations was from March to mid-May (Julian dates 60–135) and early June to mid-July (Julian dates 160–200), respectively (figs 1 and 2). At both locations, *Leucopis* spp. larvae were collected during periods when both *A. tsugae* progrediens and sistens eggs were present. Although at the St. Paul, OR location, *Leucopis* spp. larvae were collected only during the latter part of the progrediens egg generation in early May 2007 (Julian dates 122–128) and not during the same period in 2008 (fig. 1). At

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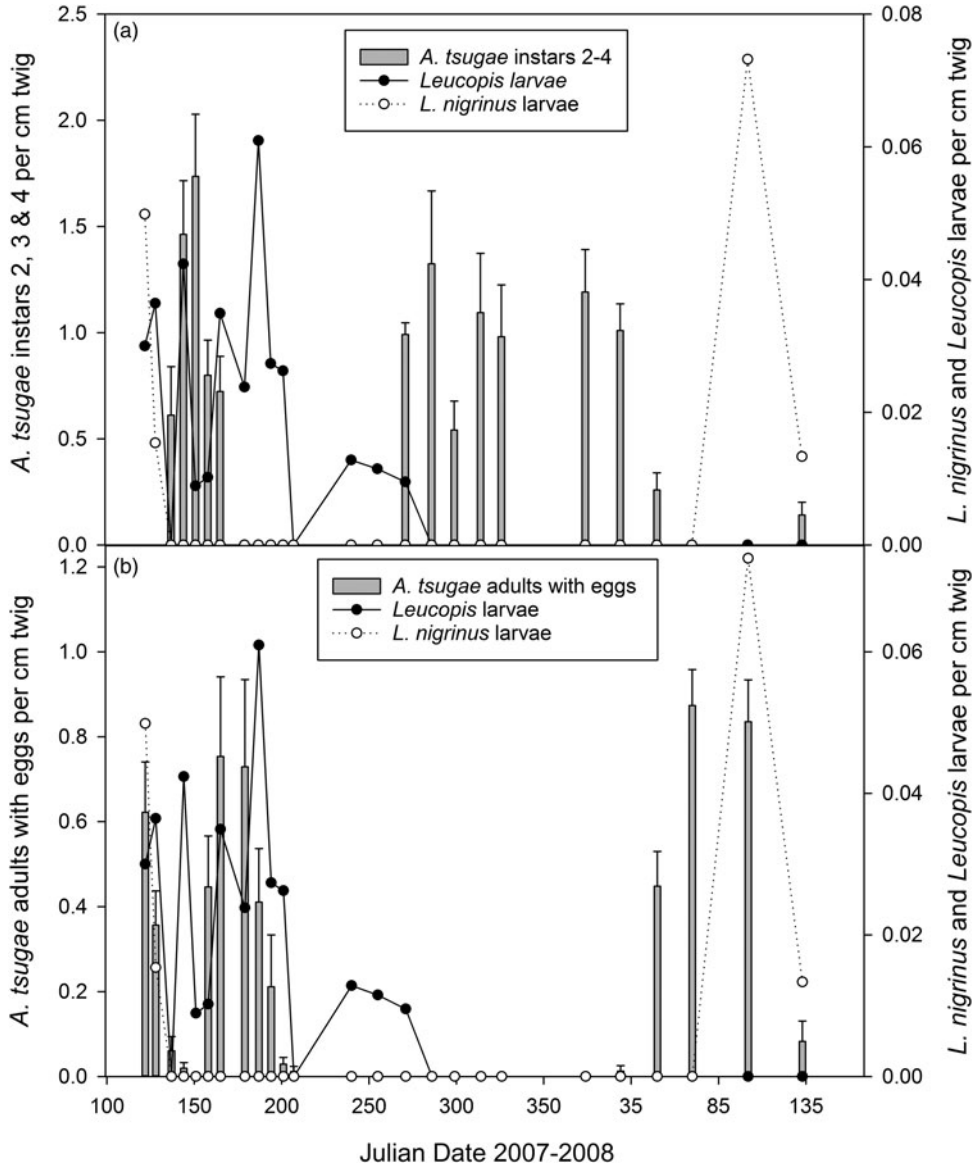

Fig. 1. Seasonal abundance of *A. tsugae* second to fourth instar nymphs (a) and adults with eggs (b) and *Leucopis* spp. and *La. nigrinus* larvae on western hemlocks in a seed orchard near St. Paul, OR.

both locations, *La. nigrinus* larvae were collected only during periods when *A. tsugae* progrediens eggs were present (figs 1 and 2).

The peak abundance of *Leucopis* spp. larvae coincided more closely with the presence of *A. tsugae* adults and eggs than nymphs at both locations (figs 1 and 2). The peak abundance of *La. nigrinus* larvae also coincided more closely with the presence of *A. tsugae* adults and eggs than nymphs at both locations (figs 1 and 2), although they were not present after early May (about Julian date 130) at either site because they pupate in the soil over the summer. Consequently, *La. nigrinus* larvae do not prey on the sistens egg stage.

At the St. Paul, OR location, the peak abundance of *La. nigrinus* larvae in 2008 was slightly higher than the peak abundance of *Leucopis* spp. larvae in 2007 (fig. 1). However, *Leucopis* spp. larvae were present for a much longer period of time and 3.5 times more *Leucopis* spp. were collected than *L. nigrinus*. A total of 42 *Leucopis* spp. (five eggs, 30 larvae, and seven puparia) were collected compared with 12 *La. nigrinus* (all larvae) at this location. A total of 3846 *A. tsugae* (125 crawlers, 2257 first instar nymphs, 527 second instar nymphs, 442 third and fourth instar nymphs, and 498 adults) were collected at this location. At the Olympia, WA location, the peak abundance of *Leucopis* spp. larvae was much greater than the peak abundance of *La. nigrinus* and, again, they were present for a much longer period of time (fig. 2). *Leucopis* spp. were collected 2.3 times more than *La. nigrinus* at this location. A total of 102 *Leucopis* spp. (93 larvae and nine puparia) were collected compared with 44 *La. nigrinus* (41 larvae and three adults). A total of 3075 *A. tsugae* (102 crawlers, 704 first instar

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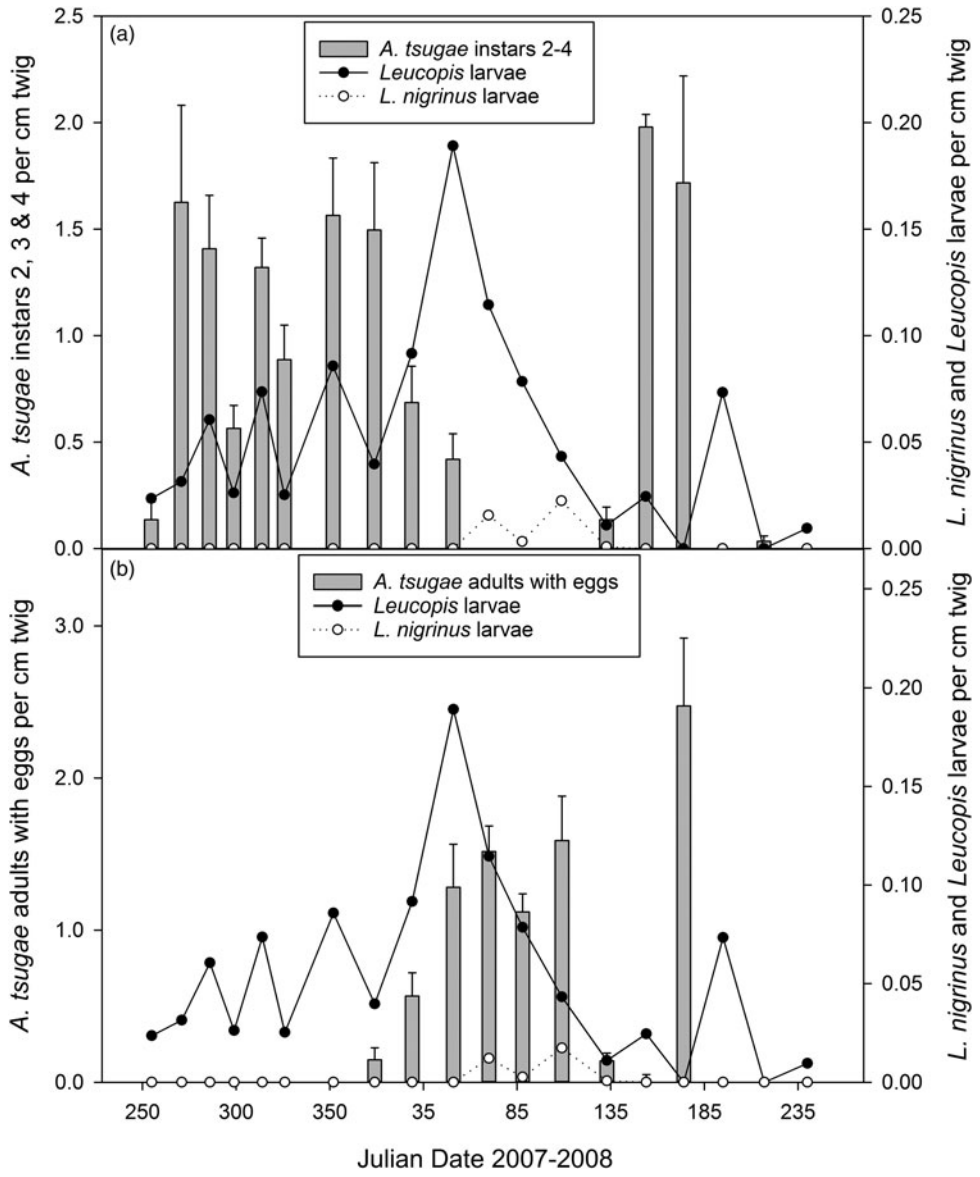

Fig. 2. Seasonal abundance of *A. tsugae* second to fourth instar nymphs (a) and adults with eggs (b) and *Leucopis* spp. and *La. nigrinus* larvae on western hemlocks in an ornamental planting in Olympia, WA.

nymphs, 517 second instar nymphs, 875 third and fourth instar nymphs, and 877 adults) were collected at this location.

Discussion

Leucopis spp. and *La. nigrinus* larvae were both most abundant at times when *A. tsugae* were present as adults and eggs. This confirms previous reports of the phenology of these species in Southwestern British Columbia, Oregon, and Washington (Zilahi-Balogh *et al.*, 2003; Kohler *et al.*, 2008; Grubin *et al.*, 2011). *Leucopis* spp. larvae were also present when all *A. tsugae* were nymphs, but *La. nigrinus* larvae were never found at those times. These results confirm that *Leucopis* spp. and *La. nigrinus* larvae feed on *A. tsugae* adults and/or eggs, but only *Leucopis* spp. larvae potentially feed on *A. tsugae* nymphs. Furthermore, *Leucopis* spp. larvae are present during both generations of *A. tsugae*, while *La. nigrinus* larvae are only found during the progrediens egg stage, i.e. mid-March to mid-May, Julian dates 70–133.

Between 2.3 and 3.5 times more *Leucopis* spp. than *La. nigrinus* were collected on the clipped branch samples. This is just about opposite of the relative abundance of the two genera of predators found in a beat sampling survey of *A. tsugae* infested hemlocks in the same region where *La. nigrinus* were 2.7 times more abundant than *Leucopis* spp. (Kohler *et al.*, 2008). However, it has been suggested previously that the beat sampling technique is likely more efficient at collecting adult *La. nigrinus* than adult *Leucopis* spp. and overestimates the abundance of the former compared with the latter (Ross *et al.*, 2011). Therefore, the numbers from the clipped branch sampling

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technique used in this study are probably more representative of the relative abundance of the two genera of predators.

Since the two species of *Leucopis* are found along with *La. nigrinus* throughout the PNW, it is likely that they will compliment rather than compete significantly with one another after release in the Eastern USA. The results of this study support previous findings that *Leucopis* spp. are important predators of *A. tsugae* in the PNW and, furthermore, are deserving of continued study as potential biological control agents for *A. tsugae* in the Eastern USA.

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