Prey Suitability and Phenology of *Leucopis* spp. (Diptera: Chamaemyiidae) Associated With Hemlock Woolly Adelgid (Hemiptera: Adelgidae) in the Pacific Northwest

SARAH M. GRUBIN,[1] DARRELL W. ROSS,[1,2] AND KIMBERLY F. WALLIN[1,3,4]

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ABSTRACT *Leucopis* spp. (Diptera: Chamaemyiidae) from the Pacific Northwest previously were identified as potential biological control agents for the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), in the eastern United States. We collected *Leucopis* spp. larvae from *A. tsugae* infested western hemlocks in Oregon and Washington and reared them on an unidentified *Pineus* spp., *Pineus strobi* (Hartig), *Adelges cooleyi* (Gillette), *Adelges piceae* (Ratzeburg), and *A. tsugae* in three no-choice tests. *Leucopis* spp. survival on *A. tsugae* was significantly higher than on *A. piceae* during the 2010 progrediens generation test and significantly higher than on *P. strobi* and *A. cooleyi* during the 2010 sistens generation test. However, across all three tests, some larvae completed development to adult on all four of the alternative adelgid species. Larvae that survived to the adult stage were identified as *Leucopis argenticollis* Zetterstedt and *Leucopis piniperda* Malloch. These results suggest that populations of *L. argenticollis* and *L. piniperda* in the Pacific Northwest may not be specific to *A. tsugae*. We also studied the phenology of *Leucopis* spp. on fourteen *A. tsugae* infested western hemlock trees in Oregon and Washington over a period of 14 mo. *Leucopis* spp. larvae were collected year-round, but highest densities coincided with the presence of progrediens and sistens eggs and adults of *A. tsugae*. There was a positive correlation between *Leucopis* spp. and *A. tsugae* abundance.

KEY WORDS *Adelges tsugae*, biological control, *Leucopis argenticollis*, *Leucopis piniperda*, prey suitability

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), accidentally was introduced into eastern North America from a location in southern Japan (Havill et al. 2006). It was first discovered near Richmond, VA in 1951 (Stoetzel 2002), but did not cause significant mortality of eastern hemlocks until the 1980s (McClure 1987, Souto et al. 1996). It has been spreading throughout the eastern United States at a mean rate of 12.5 km/yr and appears to be limited only by cold temperatures (Shields and Cheah 2005, Evans and Gregoire 2006, Morin et al. 2009). It is now found from northern Georgia to southern Maine (Anonymous 2009) causing high levels of mortality to eastern and Carolina hemlocks, *Tsuga canadensis* (L.) Carriére and *Tsuga caroliniana* Engelmann, respectively (Orwig and Foster 1998, Eschtruth et al. 2006, Faulkenberry et al. 2009).

Although insecticide applications are effective for suppressing *A. tsugae* populations, particularly in urban and horticultural settings, their use in forests is limited by accessibility, potential nontarget effects, and other ecological and economic concerns (Cowles 2009, Dilling et al. 2009, Ford et al. 2010). Consequently, a classical biological control program was initiated in 1992 with the intent of providing long-term, region-wide suppression of *A. tsugae* populations (McClure 2001, Cheah et al. 2004). Because parasitoids of adelgids are uncommon, if they exist at all, biological control efforts have focused primarily on predators (Wilson 1938, Montgomery and Lyon 1996, Yu et al. 2000, Zilahi-Balogh et al. 2002) and, to a smaller extent, entomopathogens (Reid et al. 2009). This program has led to the release of three coleopteran predator species, *Sasajiscymnus tsugae* (Sasaji and McClure) (Coccinellidae) from Japan, *Scymnus sinuanodulus* Yu and Yao (Coccinellidae) from China, and *Laricobius nigrinus* Fender (Derodontidae) from western North America (Cheah et al. 2004, Mausel et al. 2010). However, none of these predators have yet provided the desired suppression of *A. tsugae* populations, and efforts have continued to identify other predator species in Asia and western North America as good candidates for release in the eastern United States (Yu et al. 2000, Zilahi-Balogh et al. 2007, Kohler et al. 2008, Yu and Montgomery 2008, Gatton et al. 2009).

[1] Department of Forest Ecosystems and Society, Oregon State University, 321 Richardson Hall, Corvallis, OR 97331–5752.
[2] Corresponding author, e-mail: darrell.ross@oregonstate.edu.
[3] The Rubenstein School of Environment and Natural Resources, The University of Vermont, 81 Carrigan Dr., Burlington, VT 05405–0088.
[4] USDA Forest Service, Northern Research Station, South Burlington, VT 05403.

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In the Pacific Northwest, Kohler et al. (2008) identified 55 species of predators representing 14 families associated with *A. tsugae* on western hemlock, *Tsuga heterophylla* (Raf.) Sargent, of which *L. nigrinus* was the most abundant, followed by two species of Chamaemyiidae (Diptera), *Leucopis argenticollis* Zetterstedt and *Leucopis piniperda* Malloch (as *Leucopis atrifacies* Aldrich) (S. Gaimari, personal communication). Both *L. argenticollis* and *L. piniperda* are known adelgid specialists (McAlpine and Tanasijtshunk 1972, Tanasijtshuk 2002), but this was the first record of either species associated with *A. tsugae* (Kohler et al. 2008). *Leucopis argenticollis* is a Holarctic species that was previously found in colonies of several *Pineus* species in Russia, India, Canada, and the United States (McAlpine and Tanasijtshunk 1972). *Leucopis piniperda* is found throughout Canada and the United States in association with several *Adelges* and *Pineus* spp. (Greathead 1995, Tanasijtshuk 2002). After the introduction of *Adelges piceae* (Ratzeburg) into North America, *L. argenticiollis* was found associated with *A. piceae* in eastern Canada (McAlpine and Tanasijtshunk 1972).

The potential value of chamaemyiids as biological control agents for adelgids has been recognized since at least the 1930s. After the accidental introduction of *Pineus pini* (Macquart) into Australia, Wilson studied the natural enemies of *P. pini* and *Pineus strobi* Hartig in England (Wilson 1938). He identified *Neoleucopis obscura* (Haliday) as the most efficient predator of these species and suggested that it should be introduced into Australia for biological control of *P. pini.* Subsequently, a number of chamaemyiid species have been considered for biological control of *Pineus* and *Adelges* species throughout the world with varying degrees of success (Rawlings 1958, Zúñiga 1985, Culliney et al. 1988, Zondag and Nuttall 1989, Mills 1990, Greathead 1995, Zilahi-Balogh et al. 2002).

Other than a few collection records, there is little published information about the biology and ecology of *L. argenticollis* and *L. piniperda.* Because these species are common and abundant associates of *A. tsugae* in the Pacific Northwest, populations from this region may be sources of additional introductions for the biological control program in the eastern United States. Our objectives were to 1) compare the suitability of *A. tsugae* and four alternative adelgid prey species for *Leucopis* spp. larvae collected from *A. tsugae* infested western hemlock, and 2) compare the phenology and abundance of *Leucopis* spp. and *A. tsugae* on western hemlock in the Pacific Northwest.

Materials and Methods

Host Suitability. *Leucopis* spp. immatures used in this study were collected from western hemlock branches infested with *A. tsugae* found at nine locations in western Washington and Oregon, ranging from Vashon, WA in the north to Portland, OR in the south (Grubin 2011). Infested branches were transported back to the laboratory and held at 3°C with the cut ends in water. *Adelges tsugae* ovisacs were examined under a dissecting microscope, and *Leucopis* spp. eggs and larvae were removed. The immature *Leucopis* spp. were used immediately for testing because there were no rearing methods or artificial diet known for these insects, although rearing procedures have been developed for aphidophagous *Leucopis* spp. (Gaimari and Turner 1996).

In addition to *A. tsugae* eggs, collected from the same sites as the *Leucopis* spp., four alternative species of prey were used in host suitability tests based on taxonomic or ecological similarity to *A. tsugae* as well as seasonal and geographic availability (Kuhlmann et al. 2005). All selected test prey were species of Adelgidae, representing both genera in the family. All test prey were field collected in the egg stage and held at 3°C until used in the feeding trials. The four alternative prey were an unidentified *Pineus* spp. collected from branches on young lodgepole pine, *Pinus contorta* Dougl. ex. Loud., located in the Deschutes National Forest near Bend, OR; *P. strobi* collected from an ornamental planting of white pine, *Pinus monticola* Dougl. ex D. Don, in Wilsonville, OR; *A. piceae* collected from Fraser fir, *Abies fraseri* (Pursh), in a Christmas tree plantation in Lane County, OR; and *Adelges cooleyi* (Gillette) collected from an ornamental Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, located on the Oregon State University campus in Corvallis, OR.

Three no-choice feeding tests were conducted; one during the 2009 *A. tsugae* sistens generation, and two in 2010, coinciding with the *A. tsugae* progediens and sistens generations. The number of *Leucopis* spp. immatures fed each alternative prey depended upon the number that could be found during each *A. tsugae* generation, and ranged from 16 to 36 (Table 1). During the 2009 sistens generation, test prey were *A. tsugae* and the unidentified *Pineus* spp.; during the 2010 progrediens generation, test prey were *A. tsugae*, *P. strobi*, *A. cooleyi*, and *A. piceae;* and during the 2010 sistens generation, test prey were *A. tsugae*, *P. strobi*, and *A. cooleyi.*

Individual *Leucopis* spp. eggs or larvae of any developmental stage were randomly assigned to each test prey as they were collected. Feeding test containers were 50-mm-diameter petri dishes with 2.5-cm holes drilled in the lid and covered with fine mesh screen. Two layers of filter paper were placed in each petri dish and moistened with de-ionized water. Petri dishes were wrapped in Parafilm (SPI Supplies, Inc., West Chester, PA) to prevent larvae from escaping. Petri dishes were held in an environmental chamber at 25°C and 60% RH in all tests, with a photoperiod of 12:12 (L:D) h in 2009 and 14:10 (L:D) h in both 2010 tests. *Leucopis* spp. larvae were monitored every 1–3 d, given fresh prey, and the layers of filter paper were replaced. Any larvae that pupariated within the first 48 h of being placed with adelgid prey were omitted from the final results. All *Leucopis* spp. larvae that survived to the adult stage were identified to species.

Phenology and Abundance. In total, 14 western hemlock trees, infested with *A. tsugae* were sampled from 10 locations in Olympia; Tacoma; and Vashon, WA; and Portland, OR between June 2009 and August

Table 1. Mean number of days survived and percent survival of *Leucopis* spp. immatures in three no-choice feeding tests

Test	Prey	*N*	Mean days survived ± SE[a]	% survival to adult[b]
2009 Sistens	*A. tsugae*	21	11.14 ± 1.97a	29a
Generation	*Pineus* spp.	20	9.05 ± 1.74a	15a
2010 Progrediens	*A. tsugae*	20	12.25 ± 1.47a	50a
Generation	*P. strobi*	18	9.72 ± 2.39ab	0
	A. cooleyi	18	11.0 ± 1.49ab	33ab
	A. piceae	16	6.13 ± 1.01b	6b
2010 Sistens	*A. tsugae*	34	12.91 ± 1.86a	21a
Generation	*P. strobi*	36	5.77 ± 0.57b	6a
	A. cooleyi	33	5.09 ± 0.47b	0

[a] Within a test, means followed by the same letter are not significantly different, by a one-way ANOVA or Tukey–Kramer HSD test.
[b] Within a test, values followed by the same letter are not significantly different by Pearson's χ^2 contingency test.

2010 (Grubin 2011). From June 2009 through February 2010, 11 infested trees from seven sites were sampled. In late February 2010, three infested trees from three additional sites were added to the sampling regime. Concurrently, one tree and site were removed from the study when that *A. tsugae* population collapsed. During the nymphal stage of *A. tsugae* sistens, from late August through February, sampling was conducted every 3–4 wk. From February through August, sampling frequency was increased to every 2 wk. On each collection date, four *A. tsugae* infested terminal shoots 6–15 cm in length were collected haphazardly from among the infested branches on individual trees at each site. Twigs were placed into floral vials with the cut ends in water and transported back to the laboratory in a cooler with ice. Samples were processed in the laboratory within 36 h of collection by using a dissecting microscope. All developmental 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. However, counts of third- and fourth-instar *A. tsugae* nymphs were pooled because they were sometimes difficult to distinguish. We did not include dead or desiccated *A. tsugae.* All live *Leucopis* spp. eggs, larvae, and puparia were counted. *Leucopis* species have three larval instars (McAlpine and Tanasijtshunk 1972, Tanasijtshunk 2002). The first instar was identifiable by size and lack of pigment. Counts of second and third instar *Leucopis* spp. larvae were pooled because they were difficult to reliably differentiate. Field collected puparia were kept on twigs in an environmental growth chamber at 25°C, 60% RH, and a photoperiod of 14:10 (L:D) h.

Statistical Analyses. Days survived and percent survival to adult were calculated for all host suitability feeding tests. Data were analyzed using a one-way analysis of variance (ANOVA) to determine the effect of prey species on total days survived for *Leucopis* spp. immatures. Data were log-transformed where necessary to correct for non-normal sample distributions to satisfy the assumptions of ANOVA. When ANOVA indicated a significant species effect, means were compared and separated using Tukey's honestly significant difference (HSD) test. Pearson's χ^2 contingency tests were used to assess differences in overall survivorship for *Leucopis* spp. based on prey species. Statistical tests were carried out using R computer programs (R Development Core Team 2010). Tests were considered significant if $P \leq 0.05$.

Mean abundance data for *Leucopis* spp. and *A. tsugae* across all collection sites were log-transformed to correct for non-normality and analyzed using a Pearson product-moment correlation. This test allowed us to assess the degree to which the two species co-vary, and the extent to which they are in synchrony (Bjørnstad et al. 1999). Statistical tests were carried out using SPSS version 19.0.0 computer programs (SPSS Inc. 2010). Tests were considered significant if $P \leq 0.05$.

Results

Host Suitability. *Leucopis* spp. larvae fed on eggs of all test prey species. Of the 247 larvae observed across all three no-choice feeding tests, 23.5% pupariated and 15.4% emerged as adult *L. argenticollis* (27) or *L. piniperda* (11). Parasitoids emerged individually from 3% of *Leucopis* spp. puparia. Parasitoids only were reared from *Leucopis* spp. larvae collected during the 2009 test and those eight parasitoids were not identified.

During the 2009 sistens generation, *Leucopis* spp. completed development to the adult stage on diets of *Pineus* spp. and *A. tsugae.* There was no significant difference in the total days survived for larvae fed *A. tsugae* or *Pineus* spp. ($F = 0.87$; df = 1,39; $P = 0.36$) (Table 1). In addition, there was no significant difference in percent survival to adult of *Leucopis* spp. larvae between the prey species ($\chi^2 = 0.45$; df = 1; $P = 0.50$) (Table 1).

During the 2010 progrediens generation, there were significant differences in total days survived for *Leucopis* spp. among the four prey species ($F = 2.87$; df = 3,68; $P = 0.04$) (Table 1). *Leucopis* spp. larvae fed *A. tsugae* lived significantly longer than those fed *A. piceae*, but there were no differences among *A. tsugae*, *A. cooleyi*, and *P. strobi* or among the three alternate prey species. *Leucopis* spp. completed development to the adult stage on diets of *A. tsugae*, *A. cooleyi*, and *A. piceae.* A 2 by 3 Pearson's χ^2 contingency test indicated a significant effect of species on percent survival to adult ($\chi^2 = 7.93$; df = 2; $P = 0.019$). Consequently, 2 by 2 Pearson's χ^2 contingency tests were performed for all pairwise comparisons. Percent survival to adult on *A. tsugae* was significantly greater than on *A. piceae*

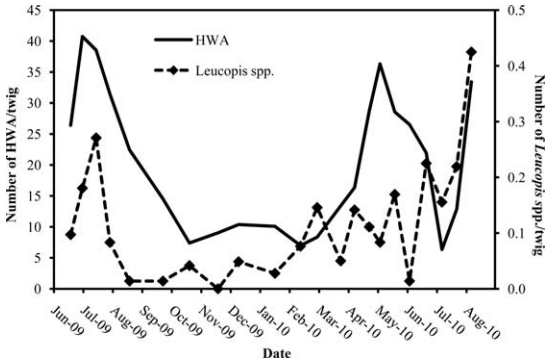

Fig. 1. Number of *Leucopis* spp. immatures and *A. tsugae* per twig (all life stages pooled) collected from *T. heterophylla* at 10 sites in Oregon and Washington between June 2009 and August 2010.

(χ^2 = 6.09; df = 1; P = 0.014), but not *A. cooleyi* (χ^2 = 0.50; df = 1; P = 0.480) (Table 1). There was no significant difference in percent survival to adult between *A. cooleyi* and *A. piceae* (χ^2 = 2.32; df = 1; P = 0.128) (Table 1).

During the 2010 sistens generation, there were significant differences in total days survived for *Leucopis* spp. among the three prey species (F = 12.12; df = 2,98; $P < 0.001$) (Table 1). *Leucopis* spp. larvae fed *A. tsugae* lived significantly longer than those fed *P. strobi* or *A. cooleyi*. *Leucopis* spp. completed development to the adult stage on diets of *A. tsugae* and *P. strobi*. There were no *Leucopis* spp. larvae that survived to the adult stage on a diet of *A. cooleyi*. There was no significant difference in percent survival to adult between *Leucopis* spp. larvae fed *A. tsugae* or *P. strobi* (χ^2 = 3.48; df = 1; P = 0.062) (Table 1).

Phenology and Abundance. In total, 132 *Leucopis* spp. immatures were collected from twig samples between June 2009 and August 2010. *Leucopis* spp. were found to be present year-round on branches infested with *A. tsugae* (Fig. 1). All *Leucopis* spp. eggs were found at the base of hemlock needles in contact with *A. tsugae* ovisacs, all *Leucopis* spp. larvae were found within *A. tsugae* ovisacs either in contact with live *A. tsugae* or within unoccupied ovisacs, and all *Leucopis* spp. puparia were found attached to twigs near *A. tsugae* ovisacs.

Data collected from twig samples confirmed that aestivating *A. tsugae* sistens nymphs resumed development in late-fall, reached the adult stage during winter, and oviposited beginning in late-winter continuing into late-spring (Fig. 2). The progrediens generation matured rapidly, ovipositing from late-spring through midsummer. Sexuparae were not observed. Aestivating sistens nymphs were present from late-summer through the fall.

Leucopis spp. larvae were collected year-round on branches infested with *A. tsugae*, except on three of 22 collection dates (Fig. 2). *Leucopis* spp. eggs were collected between March and early August, and *Leucopis* spp. puparia were collected from February through early August. Between June 2009 and August 2010, 87 *Leucopis* spp. individuals successfully were reared to the adult stage in the laboratory and identified to species. Of these, 59.8% were *L. argenticollis* and 40.2% were *L. piniperda*. Peaks in abundance of *A. tsugae* occurred on 6 July 2009, 5 May 2010, and 6 August 2010 (Fig. 1). Highest abundance of *Leucopis* spp. also occurred at about the same times (Fig. 1). Furthermore, highest abundance of *Leucopis* spp. larvae occurred on 6 July 2009, during April and May 2010, and on 7 July 2010 (Fig. 2). These times of peak abundance coincided with the presence of both progrediens and sistens eggs and adults of *A. tsugae* (Fig. 2). A Pearson product-moment correlation coefficient indicated a positive correlation in abundance between *A. tsugae* and *Leucopis* spp. (r = 0.44; n = 22; P = 0.04).

Discussion

All *Leucopis* spp. larvae collected in the field during this study that survived to the adult stage and could be

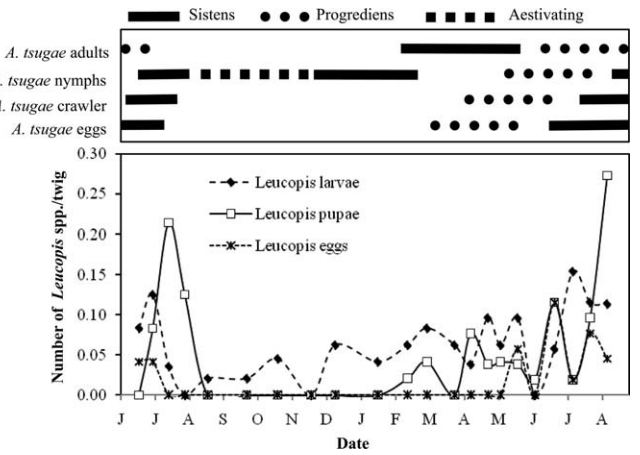

Fig. 2. Number of *Leucopis* spp. per twig collected from *A. tsugae* infested *T. heterophylla* at 10 sites in Oregon and Washington from June 2009 to August 2010. *A. tsugae* life cycle is based on observations across all sites.

identified to species ($n = 125$) were either *L. argenticollis* (63.2%) or *L. piniperda* (36.8%). In a previous study using the same or nearby collection sites, all *Leucopis* spp. immatures that survived to the adult stage were either *L. argenticollis* (87%) or *L. piniperda* (as *L. atrifacies*) (13%) (Kohler et al. 2008). Based on the results of both studies, it appears that *L. argenticollis* is the predominant species of chamaemyiid associated with *A. tsugae* in western Oregon and Washington. Until reliable methods for identifying immature *Leucopis* spp. become available or methods of rearing colonies of individual species are developed, studies such as ours will not be able to distinguish species a priori.

Across all three no-choice feeding tests, mean days survived and percent survival to the adult stage for *Leucopis* spp. was highest for larvae fed *A. tsugae*, although the differences were not always statistically significant. Across tests, mean days survived were consistent for *Leucopis* spp. larvae fed *A. tsugae*, ranging from 11.14 during the 2009 sistens generation to 12.91 during the 2010 sistens generation (Table 1). Mean days survived were considerably lower for *P. strobi* and *A. cooleyi* during the 2010 sistens generation compared with the 2010 progrediens generation (Table 1). Also, percent survival of *A. cooleyi* was 33% during the 2010 progrediens generation compared with 0% during the 2010 sistens generation. The variability in survival of *Leucopis* spp. larvae on the alternative prey among the tests further suggests that they were less suitable hosts. These tests showed that while *Leucopis* spp. feeding on *A. tsugae* may live longer and survive to the adult stage at a higher rate than those feeding on other adelgid species, the alternative prey also appear to be suitable for growth and development.

Although evidence of a preference for *A. tsugae* among *Leucopis* spp. was apparent in the feeding tests, it is important to note that all larvae used were field caught on *A. tsugae.* Prior experience of predators can act as a confounding factor in host suitability studies, reducing predator response to alternative prey, even when the preferred host is not present (Van Driesche and Murray 2004), such as in our no-choice tests. In addition, because we used field caught larvae and there are no characters to easily distinguish these species of *Leucopis* in the larval stage, we were unable to determine the species or control for the exact age of each larva before testing. Generally, when assessing host suitability for predators, it is advisable to reduce bias in favor of the target species induced by experience, by rearing and maintaining predators on a diet of species other than the target species or on an artificial diet (Withers and Browne 2004). Unfortunately, this was beyond the scope of our study.

The fact that *Leucopis* spp. associated with *A. tsugae* in the Pacific Northwest are capable of feeding and completing development on other adelgid species has both negative and positive implications for their use in the biological control program in the eastern United States. Based on published collection records, both species of *Leucopis* that have been found associated with *A. tsugae* are adelgid specialists (McAlpine and Tanasijtshunk 1972, Tanasijtshuk 2002, Kohler et al. 2008). Consequently, the only nontarget insects they are likely to feed on in the East would be other adelgid species. If they were to do so, they could potentially compete and interfere with endemic predators of those other species. However, both species already are present in eastern North America (McAlpine and Tanasijtshunk 1972, Tanasijtshuk 2002). Furthermore, there are no documented records of introduced chamaemyiids interfering with endemic predators in areas where they have been released for biological control of adelgids in other parts of the world (Mitchell and Wright 1967, Harris and Dawson 1979, Culliney et al. 1988, Mills 1990, Humble 1994, Greathead 1995). There was speculation that the introduction of *Neoleucopis obscura* (Haliday) to control *A. piceae* in eastern Canada reduced the abundance of a native predator, *Leucopina americana* (Malloch), but that was never confirmed or quantified (Brown and Clark 1957). Alternatively, one benefit of a wider host range is that if the adelgid species from the Pacific Northwest are able to readily switch between prey species in the field, this could allow them to spread more quickly and survive in areas with low populations of *A. tsugae* in the East.

Although *L. argenticollis* and *L. piniperda* are reported to occur in eastern North America, neither species has been collected from *A. tsugae* infested trees in that region. However, Wallace and Hain (2000) did collect small numbers of an unidentified *Leucopis* species from *A. tsugae* infested hemlocks at two sites in Virginia. Although these species may be present throughout North America, populations in the East and West are widely separated geographically, appear to have different host preferences, and are likely to be genetically distinct populations. Therefore, further evaluation and release of western *Leucopis* spp. in the East could contribute to biological control of the introduced *A. tsugae* populations.

The results from our field sampling indicate that *Leucopis* spp. are most abundant from February through early August when *A. tsugae* adults and eggs are present (Figs. 1 and 2). Between February and August 2009, there were three peaks in egg abundance and four peaks in pupal abundance. These peaks might reflect *Leucopis* spp. generations or differences in the life cycles between the species, or they may be an artifact of relatively low *Leucopis* spp. numbers and our sampling intensity. From mid-August until February, generally coinciding with the nymphal stages of *A. tsugae* sistens, only *Leucopis* spp. larvae were collected, and densities were lower than other times of the year. During much of this time, the *Leucopis* spp. larvae must have been feeding on aestivating sistens nymphs or they were in a dormant state themselves. In a previous study in the same geographic area, Kohler et al. (2008) reported similar seasonal patterns even though they used a longer sampling interval. In that study, there were one or two peaks in *Leucopis* spp. larval abundance during the spring and early summer depending upon location, suggesting at most two generations per year. Furthermore, they found that *L. argenticollis* that pupated in the fall did not

emerge for 4 mo, but *L. argenticollis* and *L. piniperda* (as *L. atrifacies*) that pupated in the spring emerged in 2–4 wk. Based on our data and the earlier study, it appears likely that *L. argenticollis*, and possibly *L. piniperda*, have at least two generations per year. However, because we were unable to identify immature *Leucopis* to species, it was not possible to distinguish exact generation times, or the relative abundance of each species at different times of the year.

Leucopis spp. are good candidates for further investigation of their potential as biological control agents of *A. tsugae* in the eastern United States. The next step in evaluating the Pacific Northwest *Leucopis* spp. will be to rear them separately to determine life cycles and ecological interactions among the species and their prey.

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