Community Response of Insects Associated with Eastern Hemlock to Imidacloprid and Horticultural Oil Treatments

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Environ. Entomol. 38(1): 53–66 (2009)

ABSTRACT The hemlock woolly adelgid, *Adelges tsugae* Annand, is an invasive species reducing the populations of eastern hemlock, *Tsuga canadensis* L. Carrière, throughout the eastern United States. Systemic imidacloprid and horticultural oil are the primary chemicals used to control infestations of this invasive pest; however, the impact of these two chemicals on nontarget canopy insects is unknown. This study was initiated in November 2005 to assess the effects of (1) imidacloprid soil drench, (2) imidacloprid soil injection, (3) imidacloprid tree injections, and (4) horticultural oil applications on multiple levels of organization (composition, overall specimen abundance and species richness, guild specimen abundance and species richness, and individual species) within the phytophagous and transient canopy insect community. Community composition differed significantly among treatments based on analysis of similarity. Mean species richness and specimen abundance were significantly reduced by one or more treatments. Soil drench applications significantly reduced species richness for the detritivore and phytophaga guilds. Furthermore, specimen abundance for species in the detritivore, fungivore, phytophaga, scavenger, and transient phytophaga guilds was significantly lower in the soil drench treatment. This trend was consistent in all insect guilds examined, with the exception of the hematophaga guild that was not significantly lower than for species on the control trees. Of the 293 species documented to be associated with eastern hemlocks, 33 species were found to be directly effected by one or more of the chemical treatments.

KEY WORDS eastern hemlock, canopy arthropods, imidacloprid, horticultural oil, hemlock woolly adelgid

Imidacloprid and horticultural oil are broad-spectrum insecticides often used to control the invasive hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), on eastern hemlock, *Tsuga canadensis* L. Carrière, from New England to the southern Appalachians. This exotic insect pest was first discovered in Richmond, VA, during the 1950s (Stoetzel 2002). Insecticides offer effective control in accessible areas ranging from a few weeks to a few years until more long-term solutions such as biological control agents can be established.

Imidacloprid is a systemic insecticide taken up by xylem and diffused into the xylem ray parenchyma cells located within the twigs (Steward et al. 1998, Tattar et al. 1998), where the hemlock woolly adelgid feeds (Young et al. 1995). It is a postsynaptic nicotinic acetylcholine receptor agonist and is recommended for control of several insect species, especially those pest species that have piercing-sucking mouthparts (Bayer 2007). Hemlock woolly adelgids that feed on treated trees often die within 24–48 h after ingestion or contact (Bai et al. 1991, Kidd and James 1991, Mullins and Christie 1995). Paraffinic oil is the active ingredient in most horticultural oils and is a refined petroleum product with a mode of action of suffocating the target pest. In a forest setting, horticultural oils are recommended for the control of several insect pests and, because of their universal mode of action, are not limited by feeding behavior (Sunoco 1994).

The broad-spectrum nature of both insecticides have been beneficial in pest management strategies for several insect pests (Dively et al. 1998, Nauen et al. 1998, Quintela and McCoy 1997, Palumbo et al. 2001, Royer et al. 2005, Smith and Krischik 1999) including the hemlock woolly adelgid (McClure 1991; Steward and Horner 1994; Cowles and Cheah 2002a, b; Doccola et al. 2003; Webb et al. 2003). However, >300 species of insects have been found in association with eastern hemlock (Wallace and Hain 2000, Lynch et al. 2006, Buck et al. 2005, Dilling 2007) before use of pesticides to control this invasive pest. These species represent a diversity of insect species that function within a specific guild structure (Dilling et al. 2007) that could potentially be affected by application of these insecticides. In addition to direct effects, such as reduced species richness and specimen abundance, effects on the phytophagous and transient guilds could have cascading indirect effects on the predatory and parasitoid guilds as a result of a reduction in available prey or

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hosts. Additionally, sublethal intoxication caused by exposure to imidacloprid may cause some species to be more susceptible to predation (Kunkle et al. 2001).

Nontarget impacts have been observed in a variety of other habitats documenting effects on carabids (Kunkle et al. 2001, Mullin et al. 2005), minute pirate bug, *Orius insidiosus Say* (Hemiptera: Anthocoridae) (Albajes et al. 2003), honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) (Suchail et al. 1999), convergent lady beetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) (Stark et al. 1995, Kaakeh et al. 1996), and aquatic insect decomposers (Kreutzweiser et al. 2007). However, the effect of imidacloprid and horticultural oil on nontarget phytophagous and transient insects associated with eastern hemlock is not known. If different application methods result in different levels of nontarget impacts, while still suppressing populations of the hemlock woolly adelgid, short- and long-term managerial strategies can be implemented that target control of the hemlock woolly adelgid while reducing the impact on nontarget species. Implementation of these strategies would be especially important in areas where biodiversity and maintenance of native species is a concern. This study was initiated to determine the effect of imidacloprid and horticultural oil on multiple levels of organization within the phytophagous and transient insect communities.

Materials and Methods

Study Sites. Eastern hemlocks (n = 30) were selected on 5 November 2005 at Indian Boundary within the Cherokee National Forest located in Monroe County in southeast Tennessee. This test was arranged in a split plot 2 by 5 factorial complete randomized block design. Three test blocks were established (35°23.787 N, 84°06.662 W, elevation: 543 m; 35°23.764 N, 84°06.732 W, elevation: 555 m; and 35°04.173 N, 84°06.268 W, elevation: 565 m). Each block contained 10 trees arranged in five tree pairs, with 1 tree in the pair treated in the fall (29–30 November 2005) and the other in the spring (16 April 2006). Each tree was marked with a numbered metal tag. Tree characteristics (height, transparency, density, crown class, diameter at breast height (dbh) foliage color, overall appearance, crown condition, and percent of tree infested with hemlock woolly adelgid (based on number of branches infested) were documented on 25–26 November 2005. Tree pairs were selected based on how close any two trees matched based on these characteristics. All three blocks are located in a shortleaf pine–oak (type 76) forest (Eyre 1980).

Insecticide Application. Five treatments per block (one tree per pair treated in the spring, one treated in the fall) were made consisting of imidacloprid tree injection, imidacloprid soil injection, imidacloprid soil drench, horticultural oil foliar spray, and an untreated control. The tree injection system (J. J. Mauget, Arcadia, CA) consisted of the Mauget 3 ml 10% imicide capsules and feeder tubes. Tree injection was applied at a rate of one capsule per 15 cm dbh, which is equal to 0.15 ml (AI)/2.5 cm dbh. A 0.4-cm drill bit was used to drill a hole to a depth of 1.2 cm at a downward angle into the root flair to penetrate xylem tissue, 20.5 cm above the ground. Feeder tubes were placed in the holes and capsules were attached to the feeder tubes. Capsules were spread evenly around the circumference of the tree. Capsules were left in place until total uptake of the compound was completed, ranging from 1 to 5 h.

Soil injection application was made using a Kioritz soil injector (Kioritz, Tokyo, Japan). Merit 75 WP (Bayer, Kansas City, MO) at a rate of 1 g (AI)/2.5 cm dbh was used at the test sites consisting of one soil injection for every 2.5 cm dbh. Soil injections were made using the basal system with injections made within 45 cm of the base of the trunk. Injections were spaced evenly around the tree at a depth of 7 cm below the soil surface with individual injections delivering 30 ml of insecticide.

The soil drench was applied using an FMC high pressure hydraulic sprayer (FMC, Jonesboro, AR). Merit 75 WP (Bayer) was applied at a rate of 1.5 g (AI)/2.5 cm dbh. The recommended dose of 50 g of Merit 75 WP was mixed with 379 liters of water for fall and spring treatments. The soil extending from the trunk to the drip line was sprayed with 125 liters of insecticide.

SunSpray horticultural oil (Sun Co., Philadelphia, PA) was applied using an FMC high pressure hydraulic sprayer (FMC). The mixture consisted of 7.57 liters (AI)/379 liters of water, in accordance with the product label to treat trees for adelgids. The tree was sprayed to runoff to ensure adequate coverage.

Sampling. Because of the diversity of feeding habits and mobility among those species associated with eastern hemlock, six sampling methods were selected to provide the opportunity to capture a wide range of insect species. Insect specimens were collected monthly using malaise traps and direct sampling (beat sheet, branch cuttings, handpicking, trunk vacuuming, and visual observations). One modified malaise trap was placed in the mid-canopy of each tree. The modified malaise trap design consisted of a 60 by 60 by 60-cm PVC pipe frame covered in No-Thrips insect screen. Secured to the traps were two collecting units, a pan (15 cm wide by 65 cm length by 12 cm deep) containing 900–1,000 ml of 50% propylene glycol and water, and a collecting cup (6 cm top diameter by 6.5 cm deep, 120 ml) containing 30–60 ml of 50% propylene glycol and water. Pulley systems were placed in each tree to allow for rapid movement of the trap in and out of the canopy for collection. Malaise trap samples were collected from April 2006 through April 2007, labeled, and taken to the laboratory for sorting.

Beat-sheet samples (four per tree) were taken at each cardinal direction by striking each branch five times with a 1-m stick in the bottom level of the canopy. Direct observation was conducted on each tree for 15 min per tree. All of the foliage in the bottom section of the canopy was visually inspected and insects hand collected on observation, as well as trunk vacuuming any insect observed on the bottom 1.5 m

of the trunk of the tree. Samples were placed in prelabeled (date collected, tree number, and collecting method) 6-dr vials filled with 75% alcohol and taken to the laboratory for sorting and identification. To assess sedentary insect species, 15-cm branch samples (four per tree), one in each of the cardinal directions, were collected monthly from a height of 1.5 m. Except for lepidopteran larvae that were placed in a petri dish with premoistened filter paper, specimens on branch samples were sealed in a prelabeled (date collected, tree number, and collecting method) plastic bag. Lepidopteran larvae were taken to the laboratory and reared to adults.

Preserving and Identification of Specimens. Specimens collected from malaise traps were placed in a new collecting cup (60 mm diameter by 65 mm deep; 120 ml vol) and labeled (date collected, tree number, and collecting method). Specimens from direct sampling were placed in prelabeled (date, tree number, cardinal direction, and collecting method), 75% alcohol vials (4 dr). Branch samples were examined under the microscope in the laboratory for insect specimens. Insect specimens collected were placed in vials filled with 75% alcohol and labeled. All specimens were processed in this manner with the exception of lepidopteran larvae that were placed in petri dishes with untreated eastern hemlock branches and allowed to complete their development into the adult stage for identification. In the laboratory, the excess propylene glycol was drained from those specimens collected from malaise traps. All specimens collected were sorted to order, family, genus, and species. For each taxon, insect specimens were sorted into 4-dr vials filled with 75% alcohol and labeled. Specimens were identified using standard keys. Specialists were contacted to identify difficult specimens. Voucher specimens were organized into Cornell drawers and incorporated into the University of Tennessee's insect museum.

Community response was assessed by examining the effect across multiple levels of organization: species richness (number of species) and composition (distribution and abundance of different taxa), specimen abundance (number of individual specimens of all species), guild species richness (number of species within a specific feeding guild), and guild specimen abundance (number of individuals within a specific guild). Insect guilds were examined across all treatments to test for any effect on the functional structure of insects associated with eastern hemlock, where functional structure represents proportional organization of guilds within a community. Species were assigned guilds based on documented feeding habits. Six insect guilds (detritivore, fungivore, hematophaga, phytophaga, scavenger, and transient phytophaga) were recognized. This project was part of a collaborative effort wherein the effects of chemical treatments on the canopy predator guild and soil arthropods were evaluated by Hakeem (2008) and Reynolds (2008), respectively. The phytophaga guild consists of insects that feed directly on eastern hemlock. The transient phytophaga guild consists of insects that feed on living plant material other than eastern hemlock. The detritivore guild consists of insects that feed on decaying organic material within the canopy of eastern hemlock, whereas scavengers consisted of those insects that feed on dead animals or insects but may also exhibit various other feeding habits. Hematophaga guild consists of insects that feed on blood, whereas the fungivore guild consists of those insects that primarily feed on fungi. All guild assignments were made based on the life stage when the insect specimen was collected.

Data Analysis. Data (collection date, collection method, tree number, block number, treatment, fall or spring application, order, family, genus, species, author, number of specimens, and guild) were entered into an Excel spreadsheet. Beat-sheet, direct observation, and branch samples were all considered direct collecting methods and were made within comparable time frames. Species richness, specimen abundance, guild species richness, and guild specimen abundance data for each tree were pooled across these direct collection methods to yield a single abundance number. Malaise traps, however, captured insect specimens throughout the month and as such, were not comparable with those data obtained from the direct collecting methods. Thus, malaise trap data were evaluated separately.

Differences in species diversity and abundance among samples representing community composition were determined using analysis of similarity (ANOSIM) on Bray-Curtis distances constructed in a resemblance matrix using PRIMER version 6 software (Clarke and Warwick 1997). Differences in samples were plotted using nonmetric multidimensional scaling (NMDS). Shapiro-Wilks W test for normality and Levene's test of homogeneity of variances were used to verify that specimen abundance, species richness, guild specimen abundance, and guild species richness data conformed to the assumptions of analysis of variance (ANOVA) before analysis using SAS (SAS Institute 2005). The assumptions of normality and homogeneity of variances were met. Differences in specimen abundance and species richness and guild specimen abundance and species richness between different treatments were analyzed using mixed model ANOVA and least significant differences (LSD) in SAS (SAS Institute 2005). ANOVA mixed model type 3 test of fixed effects was used to determine interactions between application timing and treatment. To determine the effect on a specific species, least squares means (lsmeans) and *t*-tests were used for each species to determine differences among treatment means. Because *t*-tests between least squares means involve multiple statistical comparisons, a Bonferroni correct α was used to avoid type I errors.

Results

Effect on Community Composition. During this study, 293 species were identified representing 226 genera, 75 families, and 9 orders. Community composition differed significantly among treatments in direct

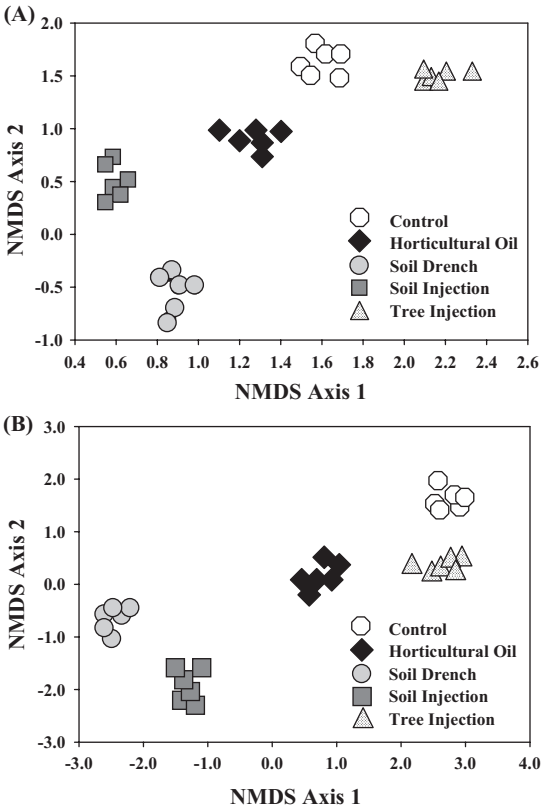

Fig. 1. Nonmetric multidimensional scaling (NMDS) ordination of 30 trees based on species and specimen abundance and Bray-Curtis similarities (stress = 0.01) for (A) direct collection samples (ANOSIM $R = 0.865$, $n = 30$, $P < 0.001$) and (B) malaise trap samples (ANOSIM $R = 0.903$, $n = 30$, $P < 0.001$). Each individual point represents a host tree. Distance between points represents the degree of dissimilarity in community composition, i.e., the further the distance, the more dissimilar the community composition.

collection samples ($R = 0.856$, $n = 30$, $P < 0.001$) and malaise trap samples ($R = 0.903$, $n = 30$, $P < 0.001$). Community composition among trees was found to be most similar among those trees within the same treatments, producing a distinctive clustering in both direct collection samples (Fig. 1A) and malaise trap samples (Fig. 1B). The soil drench cluster showed that all trees treated with a soil drench to be the most dissimilar in community composition when compared with trees treated with a soil injection, tree injection, horticultural oil, and the control in both direct collection and malaise trap samples. The potential long-term impact of this shift in community composition remains unknown.

Effect on Specimen Abundance and Species Richness. Specimen abundance (number of individual specimens of all species) was significantly lower (LSD test; $P < 0.05$) in the soil drench treatment than the control, horticultural oil, soil injection, and tree injection treatments in both direct collection (Fig. 2A) and malaise trap samples (Fig. 2B). Although specimen abundance was not significantly different (LSD test; $P > 0.05$) among the horticultural oil, soil injection, and tree injection treatments, specimen abundance was significantly lower (LSD test; $P < 0.05$) in those treatments than that recorded for the control in both direct collection and malaise trap samples. No significant interaction for direct collection samples ($F = 0.28$; df = 4,18; $P > 0.05$) and malaise trap samples ($F = 0.35$; df = 4,18; $P > 0.05$) between application time, treatment, and specimen abundance was documented. The timing of application had no significant effect on mean specimen abundance for direct collection samples ($F = 0.09$; df = 1,18; $P > 0.05$) and malaise trap samples ($F = 0.05$; df = 1,18; $P > 0.05$) by treatment. Species richness (number of species) was significantly lower in both direct collection samples (LSD test; $P < 0.05$) and malaise trap samples (LSD test; $P < 0.05$) in the soil drench treatment compared with the other treatments, whereas no significant differences in species richness in both direct collection samples (LSD test; $P > 0.05$) and malaise trap (LSD test; $P > 0.05$) samples occurred among the remaining treatments (Fig. 3). No significant interaction for direct collection ($F = 0.32$; df = 4,18; $P > 0.05$) and malaise trap samples ($F = 0.41$; df = 4,18; $P > 0.05$) between application time, treatment, and species richness was found. The timing of the application had no significant influence on species richness for either direct collection ($F = 1.32$; df = 1,18; $P > 0.05$) or malaise trap ($F = 1.08$; df = 1,18; $P > 0.05$) samples.

Overall, mean specimen abundance and species richness were effected by soil drench treatments. The effect of the soil drench may be caused by the higher concentration of imidacloprid translocated throughout the tree (Dilling 2007). Interestingly, time of the application, regardless of season, did not have a major effect on mean specimen abundance or species richness for the soil drench treatment.

Effect on Guild Specimen Abundance and Species Richness. Detritivore and fungivore guild specimen abundance (number of individuals within a specific guild) was significantly lower in the soil drench application compared with other treatments in both direct collection (LSD test; $P < 0.05$; Fig. 4A) and malaise trap samples (LSD test; $P < 0.05$; Fig. 4B and C). Guild species richness (number of species within a specific feeding guild) was significantly lower (LSD test; $P < 0.05$) for detritivore guild specimens in those trees treated with a soil drench compared with other treatments in both direct collection (Fig. 5A) and malaise trap (Fig. 5B) samples. Although no significant differences (LSD test; $P > 0.05$) were noted in guild species richness for fungivore species among treatments in the malaise trap samples, guild species richness was significantly lower (LSD test; $P < 0.05$) in trees treated with a soil drench or soil injection in direct collection samples (Fig. 5A).

In the hematophaga guild, neither specimen abundance nor species richness was significantly different among treatments for direct collection (LSD test; $P > 0.05$; Figs. 4A and 5A) and malaise trap (LSD test; $P > 0.05$; Figs. 4B and 5B) samples. The phytophaga guild

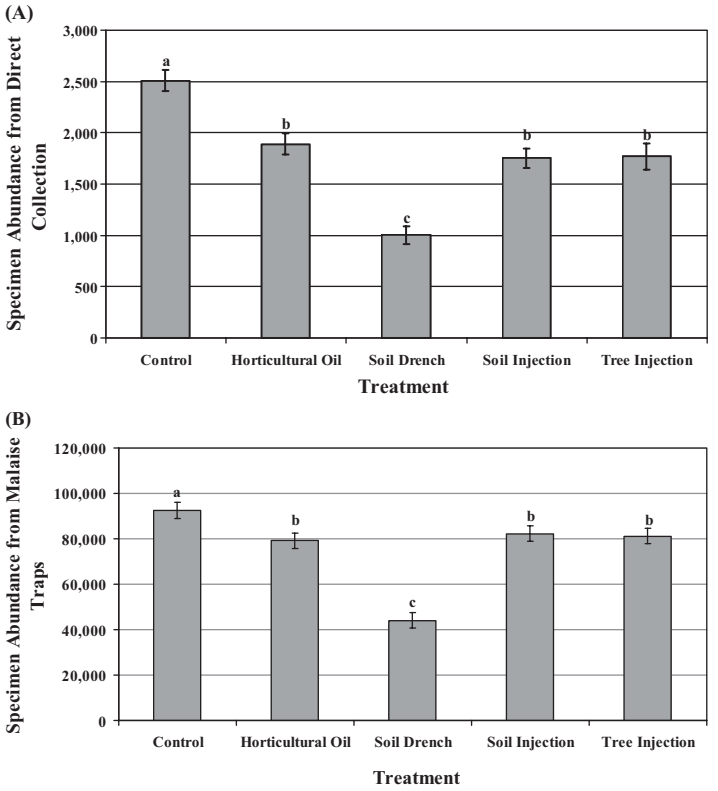

Fig. 2. Mean specimen abundance (mean number of individuals ± SE) for treatments for direct collection samples (A) and malaise trap samples (B). Means ($n = 6$) followed by the same letter are not significantly different (LSD test; $P > 0.05$).

specimen abundance was significantly lower in the soil drench and soil injection treatments in both direct collection (LSD test; $P < 0.05$; Fig. 4A) and malaise trap (LSD test; $P < 0.05$; Fig. 4B) samples compared with the control, horticultural oil, and tree injection treatments. No significant differences (LSD test; $P >$

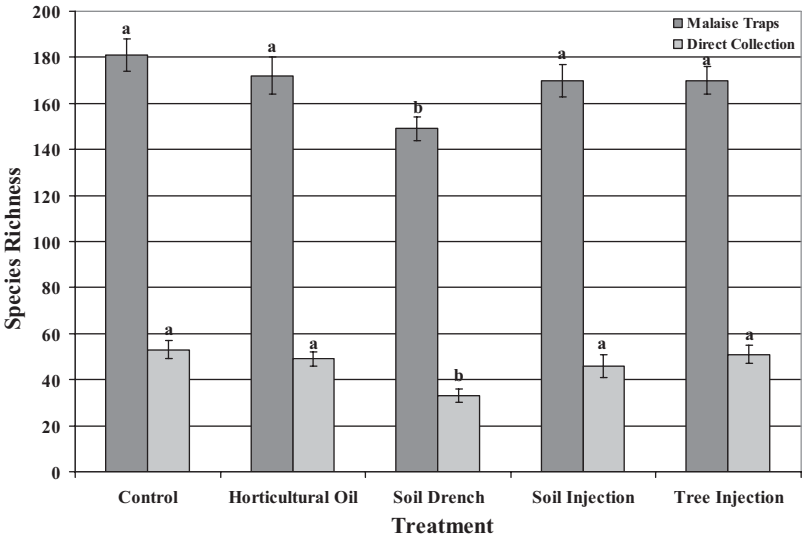

Fig. 3. Mean species richness (mean number of species ± SE) for treatments for direct collection samples and malaise trap samples. Means followed by the same letter within the same sampling regimen are not significantly different (LSD test; $P > 0.05$).

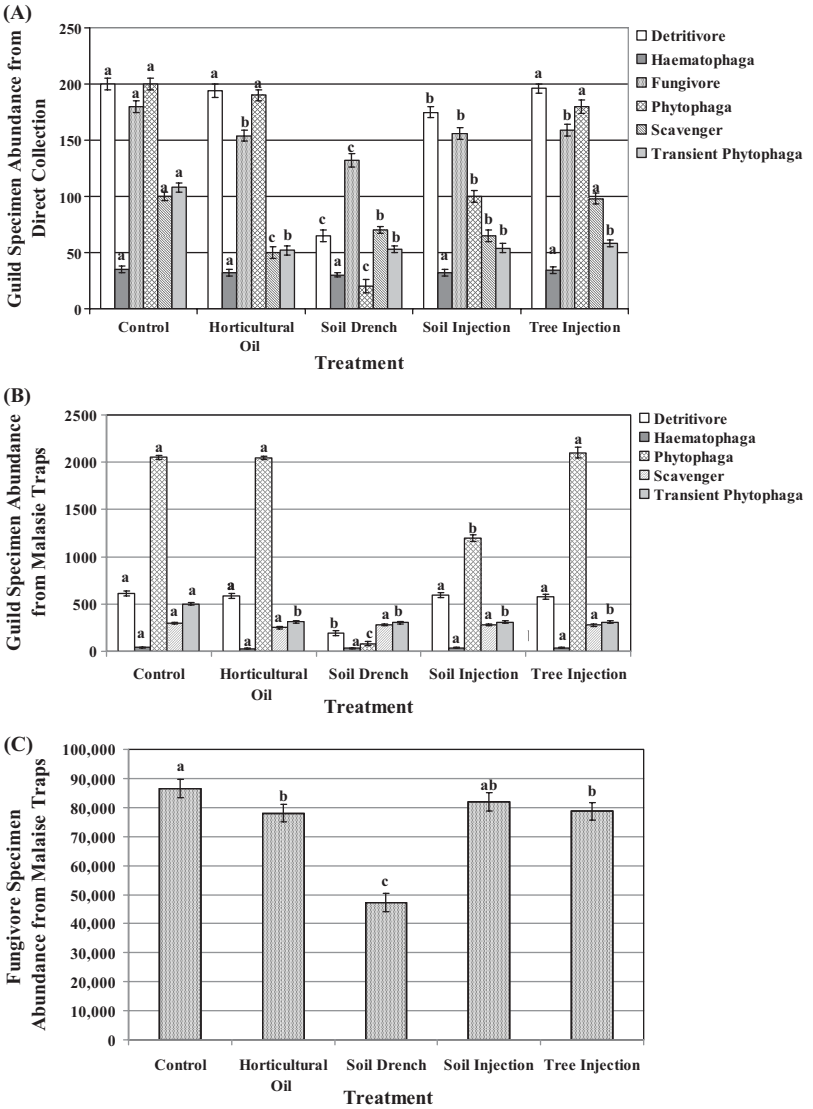

Fig. 4. Mean guild specimen abundance (mean number of individuals within a specified guild ± SE) for treatments from direct collection samples (A), malaise trap samples (B), and malaise trap samples (fungivore guild) (C). Means (n = 6) followed by the same letter within the same guild are not significantly different (LSD test; $P > 0.05$).

0.05) were noted among the horticultural oil and tree injection treatments compared with the control for both direct collection and malaise trap samples. Phytophaga guild species richness was also significantly lower (LSD test; $P < 0.05$) on those trees treated with a soil drench than in any other treatment in both direct collection (Fig. 5A) and malaise trap (Fig. 5B) samples. Transient phytophaga guild specimen abundance did not differ significantly (LSD test; $P > 0.05$) on chemically treated trees in direct collection and malaise trap samples but was significantly lower (LSD test; $P < 0.05$) than abundance noted on the control trees in both direct collection (Fig. 4A) and malaise trap (Fig. 4B) samples. The transient phytophaga guild was significantly lower (LSD test; $P < 0.05$) in species richness across chemical treatments in direct collection (Fig. 5A) and malaise trap samples (Fig. 5B).

Scavenger guild specimen abundance was significantly lower (LSD test; $P < 0.05$) on those trees treated with horticultural oil than on trees in any other treatment in direct collection samples (Fig. 4A). Scavenger guild specimen abundance on those trees treated with soil drench and soil injection did not differ significantly (LSD test; $P > 0.05$) but were lower than on the tree-injected and control trees in direct collection samples. The scavenger guild showed no significant differences (LSD test; $P > 0.05$) in specimen abundance across treatments in malaise trap samples or in species richness across treatments in direct collection samples (LSD test;

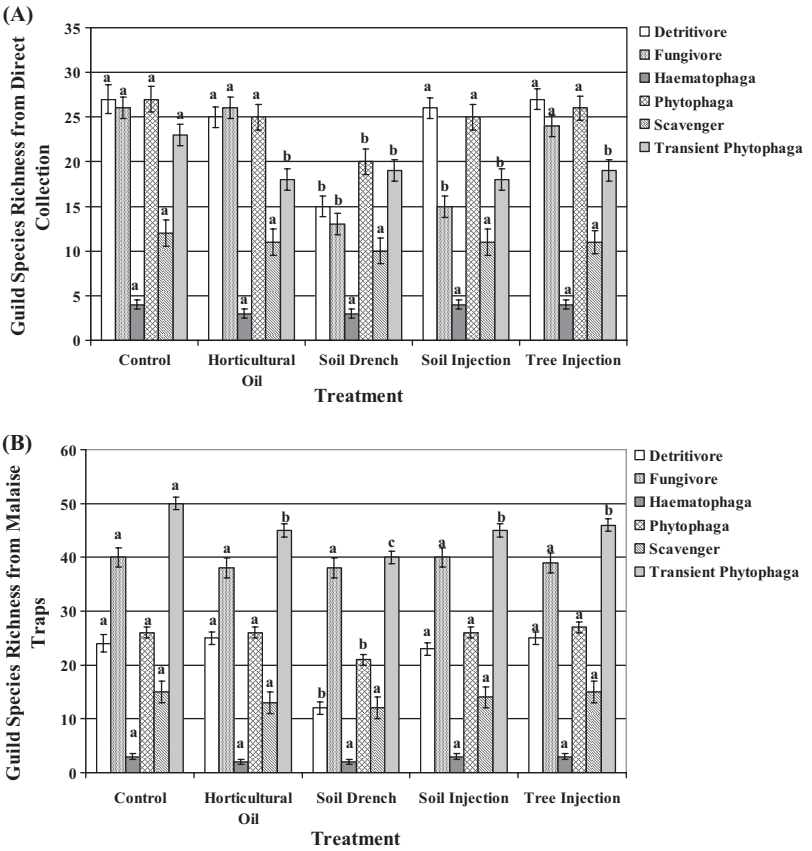

Fig. 5. Mean guild species richness (mean number of species ± SE) for treatments from direct collection samples (A) and malaise trap samples (B). Means ($n = 6$) followed by the same letter within the same guild are not significantly different (LSD test; $P > 0.05$).

$P > 0.05$; Fig. 5A) and malaise trap samples (LSD test; $P > 0.05$; Fig. 5B).

No significant difference in guild specimen abundance was noted for timing of the application for detritivore ($F = 0.04$; df = 1,18; $P > 0.05$), fungivore ($F = 0.32$; df = 1,18; $P > 0.05$), hematophaga ($F = 0.06$; df = 1,18; $P > 0.05$), phytophaga ($F = 0.18$; df = 1,18; $P > 0.05$), scavenger ($F = 0.09$; df = 1,18; $P > 0.05$), and transient phytophaga ($F = 0.23$; df = 1; $P > 0.05$) or for the interaction between time of application and treatment method for the detritivore ($F = 0.48$; df = 4,18; $P > 0.05$), fungivore ($F = 0.31$; df = 4,18; $P > 0.05$), hematophaga ($F = 0.18$; df = 4,18; $P > 0.05$), phytophaga ($F = 0.48$; df = 4,18; $P > 0.05$), scavenger ($F = 0.31$; df = 4,18; $P > 0.05$), and transient phytophaga ($F = 0.06$; df = 4,18; $P > 0.05$) in direct collection samples. Also, no significant difference in guild specimen abundance for malaise samples was noted for timing of the applications for detritivore ($F = 0.04$; df = 1,18; $P > 0.05$), fungivore ($F = 0.48$; df = 1,18; $P > 0.05$), hematophaga ($F = 0.06$; df = 1,18; $P > 0.05$), phytophaga ($F = 0.22$; df = 1,18; $P > 0.05$), scavenger ($F = 0.08$; df = 1,18; $P > 0.05$), and transient phytophaga ($F = 0.37$; df = 1; $P > 0.05$), or for the interaction between time of application and treatment method for the detritivore ($F = 0.46$; df = 4,18; $P > 0.05$), fungivore ($F = 0.39$; df = 4,18; $P > 0.05$), hematophaga ($F = 0.17$; df = 4,18; $P > 0.05$), phytophaga ($F = 0.69$; df = 4,18; $P > 0.05$), scavenger ($F = 0.48$; df = 4,18; $P > 0.05$), and transient phytophaga ($F = 0.07$; df = 4,18; $P > 0.05$).

No significant differences in guild species richness were noted for time of the application for detritivore ($F = 0.05$; df = 1,18; $P > 0.05$), fungivore ($F = 0.45$; df = 1,18; $P > 0.05$), hematophaga ($F = 0.10$; df = 1,18; $P > 0.05$), phytophaga ($F = 3.15$; df = 1; $P > 0.05$), scavenger ($F = 0.08$; df = 1,18; $P > 0.05$), or transient phytophaga ($F = 1.11$; df = 1,18; $P > 0.05$) guilds or for the interaction between time of the application and treatment method for the detritivore ($F = 0.52$; df = 4,18; $P > 0.05$), fungivore ($F = 0.38$; df = 4,18; $P > 0.05$), hematophaga ($F = 0.07$; df = 4,18; $P > 0.05$), phytophaga ($F = 0.75$; df = 4,18; $P > 0.05$), scavenger ($F = 0.47$; df = 4,18; $P > 0.05$), or transient phytophaga ($F = 0.55$; df = 4,18; $P > 0.05$) guilds in direct collection samples.

This same trend was noted for malaise trap collections. No significant differences in guild species richness were noted for time of the application for detritivore ($F = 0.09$; df = 1,18; $P > 0.05$), fungivore ($F = 0.51$; df = 1,18;

Table 1. Lepidopteran and psocopteran species potentially effected by insecticide treatment from direct collection samples

Order	Family	Genus	Species	Author	Treatment	Mean ± SD[a]
Lepidoptera	Gelechiidae	*Coleotechnites*	*apicitripunctella*	(Clemens)	HO	15.11 ± 6.53a
					CO	15.39 ± 6.42a
					SD	1.33 ± 0.49b
					SI	8.06 ± 2.34a
					TI	15.94 ± 7.07a
Lepidoptera	Geometridae	*Caripeta*	*divisata*	Walker	HO	9.88 ± 2.20a
					CO	9.88 ± 2.19a
					SD	1.44 ± 0.53b
					SI	4.15 ± 2.34c
					TI	9.11 ± 3.20a
Lepidoptera	Geometridae	*Cladara*	*limitaria*	(Walker)	HO	12.00 ± 0.95a
					CO	12.75 ± 2.18a
					SD	1.29 ± 0.49b
					SI	6.08 ± 1.51b
					TI	13.08 ± 1.44a
Lepidoptera	Geometridae	*Ectropis*	*crepuscularia*	(Denis & Schiffermüller)	HO	18.69 ± 14.9a
					CO	21.44 ± 8.13a
					SD	1.62 ± 0.11c
					SI	10.94 ± 4.76b
					TI	20.67 ± 6.29a
Lepidoptera	Geometridae	*Eufidonia*	*notataria*	(Walker)	HO	30.17 ± 6.96ab
					CO	46.11 ± 10.1a
					SD	1.83 ± 0.79c
					SI	24.67 ± 7.44c
					TI	43.78 ± 9.38b
Lepidoptera	Geometridae	*Eupithecia*	*lariciata*	(Freyer)	HO	10.73 ± 1.93a
					CO	10.97 ± 1.43a
					SD	1.50 ± 0.52b
					SI	4.70 ± 1.42b
					TI	11.43 ± 1.36a
Lepidoptera	Geometridae	*Eupithecia*	*palpata*	Packard	HO	11.30 ± 1.18a
					CO	11.80 ± 1.16a
					SD	1.00 ± 0.00c
					SI	4.60 ± 1.33b
					TI	11.87 ± 1.25a
Lepidoptera	Geometridae	*Hydriomena*	*divisaria*	(Walker)	HO	9.88 ± 2.20a
					CO	9.88 ± 2.19a
					SD	1.44 ± 0.53b
					SI	4.15 ± 2.34c
					TI	9.11 ± 3.20a
Lepidoptera	Geometridae	*Hypagyrtis*	*piniata*	(Packard)	HO	13.11 ± 1.23a
					CO	13.00 ± 0.97a
					SD	1.17 ± 0.38b
					SI	5.22 ± 1.31c
					TI	13.39 ± 1.42a
Lepidoptera	Geometridae	*Lambdina*	*fiscellaria fiscellaria*	(Guenée)	HO	23.33 ± 3.63a
					CO	25.75 ± 2.67a
					SD	1.58 ± 0.51c
					SI	12.25 ± 2.93b
					TI	24.42 ± 2.19a
Lepidoptera	Geometridae	*Macaria*	*fissinotata*	(Walker)	HO	22.90 ± 5.40a
					CO	24.27 ± 5.99a
					SD	1.63 ± 0.49c
					SI	12.47 ± 3.96b
					TI	25.33 ± 5.33a
Lepidoptera	Geometridae	*Macaria*	*signaria dispuncta*	(Walker)	HO	18.47 ± 6.05a
					CO	19.13 ± 6.20a
					SD	1.47 ± 0.51b
					SI	7.80 ± 3.72b
					TI	18.70 ± 7.73a
Lepidoptera	Geometridae	*Nepytia*	*canosaria*	(Walker)	HO	12.33 ± 1.53a
					CO	12.17 ± 1.72a
					SD	1.08 ± 0.29c
					SI	6.78 ± 1.66b
					TI	12.72 ± 1.78a
Lepidoptera	Geometridae	*Protoboarmia*	*porcelaria*	(Guenée)	HO	15.75 ± 2.63ab
					CO	14.00 ± 2.37b
					SD	1.42 ± 0.51d
					SI	7.25 ± 1.14c
					TI	19.74 ± 2.45a

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Table 1. Continued

Order	Family	Genus	Species	Author	Treatment	Mean ± SD[a]
Lepidoptera	Lymantriidae	*Dasychira*	*plagiata*	(Walker)	HO	10.08 ± 3.99a
					CO	10.90 ± 0.88a
					SD	1.00 ± 0.00b
					SI	4.83 ± 0.00b
					TI	11.33 ± 1.07a
Lepidoptera	Noctuidae	*Elaphria*	*versicolor*	(Grote)	HO	23.60 ± 1.81a
					CO	23.20 ± 1.45a
					SD	2.00 ± 0.63c
					SI	15.60 ± 3.15b
					TI	24.23 ± 1.91a
Lepidoptera	Noctuidae	*Feralia*	*comstocki*	(Grote)	HO	22.92 ± 3.56a
					CO	23.21 ± 1.79a
					SD	1.53 ± 0.52c
					SI	13.88 ± 3.69b
					TI	24.17 ± 2.66a
Lepidoptera	Noctuidae	*Feralia*	*jocosa*	(Guenée)	HO	8.72 ± 1.45a
					CO	8.72 ± 2.11a
					SD	1.00 ± 0.00c
					SI	4.22 ± 3.21b
					TI	8.94 ± 1.55a
Lepidoptera	Noctuidae	*Lithophane*	*innominata*	(Smith)	HO	7.67 ± 2.10a
					CO	6.92 ± 2.27a
					SD	1.00 ± 0.00b
					SI	2.92 ± 2.35a
					TI	6.83 ± 2.37a
Lepidoptera	Noctuidae	*Morrisonia*	*confusa*	Hübner	HO	6.72 ± 1.58a
					CO	6.44 ± 1.59a
					SD	1.00 ± 0.04c
					SI	1.56 ± 0.65b
					TI	6.47 ± 1.92a
Lepidoptera	Noctuidae	*Morrisonia*	*latex*	(Guenée)	HO	5.78 ± 1.17a
					CO	6.22 ± 1.26a
					SD	1.00 ± 0.00b
					SI	1.67 ± 0.69b
					TI	6.06 ± 1.26a
Lepidoptera	Noctuidae	*Panthea*	*acronyctoides*	(Walker)	HO	22.83 ± 1.98a
					CO	23.11 ± 1.57a
					SD	3.33 ± 2.27c
					SI	11.44 ± 1.98b
					TI	23.22 ± 1.83a
Lepidoptera	Noctuidae	*Polypogon*	*cruralis*	(Walker)	HO	12.14 ± 1.42a
					CO	12.17 ± 1.34a
					SD	1.00 ± 0.00c
					SI	5.17 ± 1.19b
					TI	11.83 ± 3.76a
Lepidoptera	Noctuidae	*Xestia*	*badicollis*	Grote	HO	22.63 ± 1.06a
					CO	23.00 ± 1.29a
					SD	2.09 ± 0.68c
					SI	14.42 ± 1.89b
					TI	23.83 ± 1.55a
Lepidoptera	Tortricidae	*Amorbia*	*humerosana*	Clemens	HO	19.67 ± 4.59a
					CO	22.33 ± 2.11a
					SD	1.50 ± 0.55c
					SI	10.67 ± 2.00b
					TI	21.50 ± 3.37a
Psocoptera	Caeciliidae	*Valenzuela*	*flavidus*	(Stephens)	HO	40.22 ± 5.55a
					CO	35.50 ± 2.57a
					SD	2.0 ± 0.54b
					SI	38.44 ± 5.77a
					TI	37.31 ± 2.76a
Psocoptera	Caeciliidae	*Xanthocaecilius*	*sommermanae*	(Mockford)	HO	14.13 ± 3.87a
					CO	15.22 ± 3.02a
					SD	2.22 ± 0.78b
					SI	14.55 ± 2.67a
					TI	15.33 ± 1.77a
Psocoptera	Peripsocidae	*Peripsocus*	*maculosus*	Mockford	HO	87.00 ± 6.77a
					CO	89.00 ± 7.12a
					SD	4.23 ± 1.32c
					SI	56.75 ± 4.55b
					TI	91.23 ± 7.98a

Continued on following page

Table 1. Continued

Order	Family	Genus	Species	Author	Treatment	Mean ± SD[a]
Psocoptera	Peripsocidae	*Peripsocus*	*subfasiatus*	(Rambur)	HO	31.00 ± 3.45a
					CO	28.45 ± 4.56a
					SD	2.13 ± 0.78b
					SI	26.88 ± 5.56a
					TI	32.22 ± 4.77a
Psocoptera	Philotarsidae	*Aaroniella*	*badonneli*	(Danks)	HO	22.3 ± 3.22a
					CO	24.34 ± 2.45a
					SD	1.22 ± 0.55b
					SI	21.44 ± 4.33a
					TI	22.9 ± 4.45a
Psocoptera	Philotarsidae	*Aeroniella*	*maculosa*	(Aaron)	HO	91.00 ± 6.67a
					CO	87.00 ± 7.32a
					SD	3.00 ± 1.34C
					SI	85.00 ± 9.34a
					TI	89.34 ± 6.44a
Psocoptera	Psocidae	*Blaste*	*opposita*	(Banks)	HO	37.23 ± 5.34a
					CO	35.76 ± 6.44a
					SD	2.22 ± 1.30b
					SI	29.33 ± 8.34a
					TI	39.34 ± 7.23a
Psocoptera	Psocidae	*Metylophorus*	*novaescotiae*	(Walker)	HO	16.56 ± 4.34a
					CO	18.23 ± 6.35a
					SD	1.07 ± 0.56b
					SI	17.56 ± 5.34a
					TI	18.34 ± 5.45a

[a] Mean no. of individuals ± SD ($n = 6$) within species grouping followed by the same letter are not significantly different based on least squares means *t*-test with a Bonferroni corrected α.

HO, horticultural oil; CO, untreated control; SD, soil drench; SI, soil injection; TI, tree injection.

$P > 0.05$), hematophaga ($F = 0.13$; df = 1,18; $P > 0.05$), phytophaga ($F = 4.32$; df = 1; $P > 0.05$), scavenger ($F = 0.17$; df = 1,18; $P > 0.05$), or transient phytophaga ($F = 1.45$; df = 1,18; $P > 0.05$) guilds or for the interaction between time of the application and treatment method for the detritivore ($F = 0.38$; df = 4,18; $P > 0.05$), fungivore ($F = 0.41$; df = 4,18; $P > 0.05$), hematophaga ($F = 0.05$; df = 4,18; $P > 0.05$), phytophaga ($F = 0.72$; df = 4,18; $P > 0.05$), scavenger ($F = 0.53$; df = 4,18; $P > 0.05$), or transient phytophaga ($F = 0.62$; df = 4,18; $P > 0.05$) guilds in malaise trap samples.

Treatment methods had varying effects on species abundance and richness within guilds and resulted in shifts in guild structure based on the type of treatment. However, the long-term direct and indirect effects of shifts in guild structure in this system are unknown.

Effect on Species. Independent *t*-tests on the differences of least squares means for the 293 insect species identified in this study indicated that 33 species were significantly effected by imidacloprid in direct collection methods (Table 1), and 7 of these 33 species were significantly effected by imidacloprid in malaise trap samples (Table 2). These 33 species belong to the phytophaga and detritivore guilds. The phytophagous species represent the lepidopteran families Gelechiidae, Geometridae, Lymantriidae, Noctuidae, and Tortricidae, whereas the detritivore species collected were psocopterans in the families Caeciliidae, Peripsocidae, Philotarsidae, and Psocidae.

The soil drench treatment had the greatest effect on all 33 insect species compared with the control and the horticultural oil treatments in both direct collection (*t*-test; $P < 0.0001$) and malaise trap (*t*-test; $P < 0.0001$) samples. For most species evaluated, no significant differences were found among those treated with horticultural oil, tree injection, and the control in direct collection (*t*-test; $P > 0.0006$) and malaise trap (*t*-test; $P > 0.0006$) samples.

Impact of Collection Methods. Direct collection and malaise trap sampling have been used as canopy collection techniques in a variety of studies (Morris 1955; Crossley et al. 1973; Ohmart et al. 1983; Majer and Recher 1988; Costa and Crossley 1991; Basset et al. 1992, 1997; Moran et al. 1994). These techniques are often biased toward the collection of certain taxa, primarily based on the mobility of the species. For example, malaise traps have been effective at collecting flying insects, especially hymenopteran and dipteran specimens (Basset et al. 1997).

In this study, malaise trap samples comprised 93% of the total specimen abundance and 58% of the species richness on eastern hemlock. The majority (89%) of specimen abundance from the malaise trap came from a variety of small dipteran species. Although collections from direct sampling represented only 7% of the total specimen abundance, it represented 42% of the species richness for insects occurring on eastern hemlock. Direct collections that consisted of beat-sheet, branch cuttings, handpicking, trunk vacuuming, and visual observations represented 26, 7, 5, 3, and 1%, respectively, of the total species richness.

Most species collected using malaise traps are highly transient in nature; however, they still enter the tree canopy and become part of the food web. As such, the importance in documenting changes in the species composition is tantamount in determining changes in the potential prey for generalist predators residing within the canopy. Direct collection techniques were

Table 2. Lepidopteran species potentially effected by insecticide treatment from malaise trap samples

Order	Family	Genus	Species	Author	Treatment	Mean ± SD[a]
Lepidoptera	Geometridae	*Eufidonia*	*notataria*	(Walker)	HO	9.86 ± 1.72a
					CO	11.34 ± 1.96a
					SD	2.34 ± 1.46c
					SI	4.42 ± 1.34b
					TI	10.36 ± 2.43a
Lepidoptera	Geometridae	*Lambdina*	*fiscellaria fiscellaria*	(Guenée)	HO	15.46 ± 3.20a
					CO	17.92 ± 2.46a
					SD	2.46 ± 1.59c
					SI	6.48 ± 1.32b
					TI	16.47 ± 1.69a
Lepidoptera	Geometridae	*Macaria*	*signaria dispuncta*	(Walker)	HO	15.42 ± 1.79a
					CO	16.73 ± 1.66a
					SD	1.42 ± 1.06c
					SI	5.72 ± 2.49b
					TI	16.44 ± 2.43a
Lepidoptera	Geometridae	*Protoboarmia*	*porcelaria*	(Guenée)	HO	12.44 ± 2.36a
					CO	13.29 ± 2.45a
					SD	2.46 ± 1.69c
					SI	5.62 ± 1.10b
					TI	13.69 ± 3.49a
Lepidoptera	Noctuidae	*Feralia*	*comstocki*	(Grote)	HO	12.46 ± 2.36a
					CO	10.32 ± 3.24a
					SD	2.46 ± 1.33c
					SI	4.56 ± 1.46b
					TI	11.22 ± 2.93a
Lepidoptera	Noctuidae	*Panthea*	*acronyctoides*	(Walker)	HO	21.46 ± 3.49a
					CO	22.55 ± 2.63a
					SD	3.49 ± 1.14b
					SI	8.44 ± 1.46b
					TI	21.52 ± 1.72a
Lepidoptera	Noctuidae	*Xestia*	*badicollis*	(Grote)	HO	12.46 ± 1.55a
					CO	13.59 ± 2.46a
					SD	3.49 ± 2.15c
					SI	9.44 ± 1.22b
					TI	13.21 ± 2.44a

[a] Mean no. of individuals ± SD ($n = 6$) within species grouping followed by the same letter are not significantly different based on least squares means t-test with a Bonferroni corrected α.

HO, horticultural oil; CO, untreated control; SD, soil drench; SI, soil injection; TI, tree injection.

effective at collecting species within all guilds and yielded several different species compared with those collected by the malaise trap. For example, beat-sheet and trunk vacuuming were the only two sampling techniques that captured specimens in the hematophaga guild. These data show the importance of using a variety of sampling methods to assess the biodiversity of arthropods in the canopy. Despite differences in abundances and species collected between the two types of sampling methodologies, similar trends resulting from the chemical treatments were recognized in both malaise trap and direct collection samples across multiple levels of organization.

Discussion

Because insects in the phytophaga guild feed directly on eastern hemlock, uptake of imidacloprid through feeding is expected for some species. Additionally, imidacloprid also works by direct contact (Elbert et al. 1998). For example, all the lepidopteran species that seemed to be effected are known to pupate in the soil, usually at the base of a tree. As such, pupae of these species could potentially come in direct contact with a soil drench application or a soil injection method, which may contribute to a reduction in numbers. Although soil injections have limited coverage initially in the soil, imidacloprid has been shown to be significantly mobile in soil column studies (Vollner and Klotz 1997, Gupta et al. 2002), as well as field studies (Felsot et al. 1998). The degree of coverage by the application method may explain the disparity between soil injection and soil drench applications wherein a lower specimen abundance occurred in the soil drench treatments compared with the soil injection treatments. The psocopteran species affected in this study are classified as detritivores feeding primarily on decaying organic material, but will also feed on decaying microfungi present on the needles (Mockford 1993). The microfungi have hyphae that penetrate the plant tissue and absorb nutrients from the plant tissue. As such, they may have the potential to uptake imidacloprid; thus, exposing feeding psocopterans to lethal concentrations of imidacloprid. However, the uptake of imidacloprid by fungi on plants has not been documented and this represents an area of future research.

Application timing (fall versus spring) did not have a significant effect on nontarget phytophagous and transient insects. This information will allow for a

broader application time period; thus, providing more flexibility to forest managers regarding when control measures can be applied with minimal nontarget effects. Significantly higher concentrations of imidacloprid were translocated in soil-drenched trees than trees treated with soil injection and tree injection (Dilling 2007). These higher concentrations of imidacloprid had the most effect on overall specimen abundance and species richness, guild specimen abundance and species richness, and specific species abundances. In most instances, the effect of tree injection on insects was similar to that of the control treatment having a minimal to no significant impact on observed species richness, species abundance, guild species richness, guild species abundance, and on specific species abundances. This minimized effect may in part be attributed to the nonuniform distribution and extremely low concentrations and residuals of imidacloprid throughout the canopy (Dilling 2007) relative to other treatment methods.

The effect of chemical treatments on individual species is evident and several species are more sensitive to soil drench and soil injection treatments than to others treatments. The lepidopteran species that are more prone to be effected in this study are polyphagous and can feed on other tree species. The psocopterans potentially effected in this study are detritivores on several hosts (Mockford 1993). In addition to these direct impacts, indirect impacts, such as the reduction of phytophaga insects, may alter the rate of nutrient turnover and other ecological processes (Janzen 1981; Schowalter et al. 1981, 1986; Erwin 1995; Stork et al. 1997; Winchester 1997). The reduction in the number of phytophaga and transient species may result in a reduction in the number of predators and parasitoids associated with this tree as the result of a lower number of available or preferred prey. A decrease in predator density was noted by Hakeem (2008) that may have resulted from a reduction in the phytophaga and transient species. However, Hakeem (2008) did note that predator populations rebound between 1 and 1.5 yr after treatment.

The differences in the effect on nontarget canopy insects for soil drench and soil injection imidacloprid concentrations may represent a threshold of tolerance for some species. However, the correlation between imidacloprid concentration and LC_{50} of nontarget insects is not known and has the potential to be highly variable among species. Also, the LC_{50} of imidacloprid on hemlock woolly adelgid is loosely correlated with existing estimates varying from 120 to 300 ppb (Cowles et al. 2006). Because of the small time frame remaining before the widespread establishment and potential dominance of the hemlock woolly adelgid on eastern hemlocks in the southern Appalachians, a better understanding of the insecticide's long-term effect on both target and nontarget species is needed to avoid the potential displacement of those native species now inhabiting the region. The available data documenting the differential effects on nontarget phytophagous and transient insects as a result of using different application methods will aid resource managers and landowners in the implementation of short and long-term managerial strategies to suppress populations of the hemlock woolly adelgid, while minimizing the effect on nontarget arthropods.

Acknowledgments

We thank D. Paulsen (Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN) for assistance with field experiments, C. Dietrich (Illinois Natural History Survey) and E. Mockford (Illinois State University) for identification of specimens, A. Reed (Statistical Consulting Center, University of Tennessee, Knoxville, TN) for statistical assistance, personnel at the Tellico Plains Ranger Station (Cherokee National Forest) for assistance in setting up the test sites, and the USDA Forest Service for financial support of this project.

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Received 27 May 2008; accepted 12 November 2008.