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Imidacloprid Movement in Soils and Impacts on Soil Microarthropods in Southern Appalachian Eastern Hemlock Stands

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Imidacloprid is a systemic insecticide effective in controlling the exotic pest *Adelges tsugae* (hemlock woolly adelgid) in eastern hemlock (*Tsuga canadensis*) trees. Concerns over imidacloprid impacts on nontarget species have limited its application in southern Appalachian ecosystems. We quantified the movement and adsorption of imidacloprid in forest soils after soil injection in two sites at Coweeta Hydrologic Laboratory in western North Carolina. Soils differed in profile depth, total carbon and nitrogen content, and effective cation exchange capacity. We injected imidacloprid 5 cm into mineral soil, 1.5 m from infested trees, using a Kioritz soil injector. We tracked the horizontal and vertical movement of imidacloprid by collecting soil solution and soil samples at 1 m, 2 m, and at the drip line from each tree periodically for 1 yr. Soil solution was collected 20 cm below the surface and just above the saprolite, and acetonitrile-extractable imidacloprid was determined through the profile. Soil solution and extractable imidacloprid concentrations were determined by high-performance liquid chromatography. Soil solution and extractable imidacloprid concentrations were greater in the site with greater soil organic matter. Imidacloprid moved vertically and horizontally in both sites; concentrations generally declined downward in the soil profile, but preferential flow paths allowed rapid vertical movement. Horizontal movement was limited, and imidacloprid did not move to the tree drip line. We found a negative relationship between adsorbed imidacloprid concentrations and soil microarthropod populations largely in the low-organic-matter site; however, population counts were similar to other studies at Coweeta.

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Eastern hemlock [*Tsuga canadensis* (L.) Carr.] is a major component of riparian forests in the southern Appalachian Mountains. As the dominant conifer in riparian areas, this species plays an important role in regulating nutrient cycling processes and climatic conditions in terrestrial and aquatic environments (Ellison et al., 2005). In the 1950s, an exotic pest, the hemlock woolly adelgid (*Adelges tsugae* Annand [Homoptera: Adelgidae])(HWA), was introduced to the mid-Atlantic region of North America from Asia. Hemlock woolly adelgid has spread throughout most of the range of eastern hemlock, moving north toward Canada and to the southern Appalachians. Hemlock woolly adelgid infestation results in slow defoliation and can result in hemlock death in 4 to 5 yr (Young et al., 1995) or up to 10 yr (Pontius et al., 2006) after infestation.

Imidacloprid (*N*-[1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl]nitramide) was the first commercially available chloronicotinyl insecticide. It was synthesized in 1985 and registered for use in 1994 (Silcox, 2002) and has proven to be an effective control of HWA in eastern hemlock trees (Steward and Horner, 1994). Imidacloprid application methods include stem injection and soil applications. Soil injection or soil drench application methods appear to be most effective in the control of HWA; however, eastern hemlock often occurs in riparian areas where stem injection is used to prevent movement of imidacloprid into nearby streams (Cowles et al., 2006). Questions remain concerning the movement of imidacloprid through forest soils in situ, especially in the high rainfall region of the southern Appalachians.

Imidacloprid is water soluble (510 mg L^{-1}), making it susceptible to leaching in soils (Gupta et al., 2002) and movement into surface waters, where significant impacts on nontarget insects are possible. For example, Kreutzweiser et al. (2008a) tested imidacloprid impacts on aquatic systems and found reduced survival of stonefly and lower leaf decomposition by leaf shredding insects. Imidacloprid can affect terrestrial organisms as well. In another study, Kreutzweiser et al. (2008b)

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Abbreviations: eCEC, effective cation exchange capacity; HWA, hemlock woolly adelgid.

examined the effects of imidacloprid applied by soil drench and found that a sensitive species of earthworm had an LC_{50} at litter layer concentrations of 5.7 mg kg^{-1} and weight loss at 3 mg kg^{-1}. These concentrations are typical for soil-applied imidacloprid and are well below the soil sorption coefficient for imidacloprid, which ranged from 15 to 40 mg kg^{-1} in unamended sandy soil and soils with added organic materials, respectively (Cox et al., 2004). Cox et al. (2004) determined the half-life of imidacloprid in soil and found that, with or without organic matter additions, $t_{1/2}$ was approximately 62 d in dark incubations and less than 90 h in light conditions.

Imidacloprid movement and retention by soils is attributed to preferential flow path movement and adsorption to soil largely controlled by soil organic matter and clay content, followed by mass flow movement (Cox et al., 2004; Cox et al., 1998b; Fernandez-Perez et al., 1998; Papiernik et al., 2006). Felsot et al. (1998) studied the movement of imidacloprid through a very fine sandy loam soil using subsurface drip irrigation application and found that initial movement of imidacloprid was along preferential flowpaths in the soil; imidacloprid was found throughout the 105-cm profile within 7 d of application. At 60 d after application, imidacloprid concentrations were more homogeneous throughout the profile (Felsot et al., 1998). Gonzalez-Pradas et al. (2002) examined imidacloprid movement after surface spray application to a greenhouse soil, made up of a 10-cm sand layer, above a clay layer on top of the native soil. Imidacloprid was detected in the sandy surface soil 1 d after application. Movement through the soil to all layers (down to 40 cm) occurred by Day 28. Application of imidacloprid using chemical irrigation methods resulted in detection in all soil layers by Day 7.

Forest soils typically have greater total C content and spatial variability in surface horizons compared with agricultural soils. Soils in the southern Appalachians typically are coarse textured and have well developed soil profiles with clay accumulation horizons in the lower profile. These factors should limit the vertical and horizontal movement of imidacloprid; however, it is unknown whether they can offset the potential impacts of high rainfall (1800–2500 mm annually) and movement along preferential flowpaths in the southern Appalachians.

Understanding soil adsorption capacity is also important for assessing the efficacy of imidacloprid application. For example, Fernandez-Perez et al. (1998) suggested that formulation of imidacloprid with lignin compounds slowed the release of the insecticide, increasing the effectiveness of the soil treatments by allowing continued uptake of the insecticide by the tree. The long-term availability and uptake of imidacloprid from the soil is evident in data presented by Cowles et al. (2006), where foliar concentrations of imidacloprid were significant 2 yr after soil application.

The objectives of this study were (i) to examine the movement and adsorption of imidacloprid in forest soils after application with the soil injection method and (ii) to quantify the impacts of imidacloprid on soil microarthropod populations.

Materials and Methods

Site Description

Study sites were located at the Coweeta Hydrologic Laboratory, a USDA Forest Service experimental forest. One of the sites was located in a low-elevation (731 m) riparian area and the other in a high-elevation (1097 m) side slope. The low-elevation site receives an average annual rainfall of 1794 mm. Soils are in the Evard series, fine-loamy, oxidic, mesic Typic Hapludults (Thomas, 1996); depth to saprolite was 50 cm. The high-elevation site receives an average annual rainfall of 2235 mm. Soils are in the Plott series, coarse-loamy, mixed, mesic Typic Haplumbrepts; depth to saprolite was greater than 90 cm. Within each site we selected five eastern hemlock trees. Four trees were treated with imidacloprid using soil injection application; they were 10 to 15 m apart and greater than 10 m from any stream. We selected study trees of a similar size (22–35 cm diam. at breast height) to ensure consistent water uptake patterns (Ford et al., 2007). One tree served as a reference within the same area, approximately 25 m from any treated tree.

Imidacloprid application methods for each tree followed the recommendation of the Forest Service, USDA (Rusty Rhea, personal communication 2008). Five feet (1.5 m) from the base of the tree, 29.5 mL of Merit solution (1.5 g active ingredient) per inch (2.54 cm) diameter at breast height was injected 5 cm into the mineral soil using a Kioritz soil injector. The cove soils in these sites had a shallow O horizon, with negligible Oa accumulation. The injection depth was 5 cm beneath the O horizon, into the high-organic-matter surface A horizon. All sample collections and lysimeter installations (described below) use the surface of the A horizon as a reference point. Soil water sample collection began 1 wk after imidacloprid application and continued for 1 yr. Around each tree, we established a circular grid to sample soil and soil water (Fig. 1). Sample collection points were located 1.0 m from the tree (inside the 1.5-m imidacloprid application circle), 2.0 m from the tree (outside the application circle), and beneath the drip line of each tree. The dripline averaged 3.4 m from the tree in the low-elevation site and 4.3 m in the high-elevation site. In each of three compass directions (120°, 240°, and 360°), lysimeter transects were installed. At each sample point, two lysimeters were installed, 20 cm into the soil and at the bottom of the B horizon, just above the saprolite layer, for a total of 18 lysimeters per tree. The 20-cm depth was within the major rooting zone, just below the A horizon in the low-elevation site and in the A horizon at the high-elevation site; the bottom of the B horizon was 50 cm in the low-elevation site and 90 cm in the high-elevation site. We used Teflon PRENART lysimeters (Prenart Equipment) to prevent lysimeter/imidacloprid interactions. Lysimeters were installed to depth at a 45° angle using a 2.54-cm soil probe. Lysimeters were connected, via Teflon tubing (covered with aluminum foil), to amber bottles to protect the imidacloprid in solution from light. Tension was applied (–0.05 MPa) to the amber bottle three times during the week of soil solution collection. Soil solution was collected each week for 6 wk after soil injection treatment; all samples were analyzed to determine imidacloprid concentration (up to 288 samples). Extreme drought made 2007 the driest year on record at the Coweeta Hydrologic Laboratory; this resulted in many missing samples during the first 6 wk after imidacloprid application. Soil solution samples were also collected during Months 3, 6, 9, and 12 after imidacloprid application (up to 576 samples). Each month, samples were collected weekly for 4

wk as described above and frozen until analysis. We established one set of lysimeters (one shallow and one deep) beneath the reference tree (not treated with imidacloprid) at each low- and high-elevation site. Sample collection intervals and procedures were the same as described above, resulting in four reference sample collections per week. Imidacloprid concentration was determined in soil solution with high-performance liquid chromatography with UV detector (Thermo Fisher Scientific, Inc.) using a Phenomenex Luna column (150 × 4.6 mm; C8(2); 5 μm) (Phenomenex Inc.) with a 1.0 mL min $^{-1}$ flow rate at 40°C, UV detection at 270 nm, and a 100-μL injection volume. Sample dilutions were made as necessary to eliminate matrix interference. Detection limits were 1 μg L^{-1} in solution.

Near each lysimeter installation, soil environmental measurements were made weekly during each soil solution collection. We measured soil moisture coincident with lysimeter sampling using time-domain reflectometry (Topp et al., 1980) in surface soils (0–20 cm) using a Campbell Scientific Hydrosense, and soil temperature was measured at 10 cm using a digital temperature probe. Within each site, we also made continuous measurement of deep soil moisture at four locations using reflectometers (Campbell Scientific) installed at locations selected to characterize changes in subsoil moisture within the area of the treated trees. Each reflectometer is comprised of a pair of 30-cm stainless steel rods inserted vertically into the soil. We installed the soil moisture probes at 20 to 50 cm at low elevation and 60 to 90 cm at high elevation. Measurement of volumetric soil water content (%) is integrated across the 30-cm depth. Soil moisture measurements were made hourly, and data were stored in an automated data logger.

To examine soil adsorption of imidacloprid, we collected soil samples 2 wk, 6 mo, and 12 mo after imidacloprid application. At each sample date, we sampled soil from two randomly selected compass directions on the circular plots around the tree at each distance (–0.5 m, 0.5 m, dripline) from the imidacloprid application line as described above. Soils were collected by depth (0–20 cm, 20–50 cm, and 50+ cm [high elevation only]), composited by tree, and analyzed for adsorbed imidacloprid concentration using acetonitrile extraction (5 g soil in 20 mL acetonitrile) (Ying and Kookana, 2004) (216 samples) followed by evaporation and dissolution in 10% methanol. This method has a reported extraction efficiency of 99% (Ying and Kookana, 2004). Soils beneath reference trees were sampled during the first sample collection only; extraction of these soils was included as a blank during extraction of each collection set. Imidacloprid concentrations in soil extracts were determined by high-performance liquid chromatography as described above; detection limits were 1 μg kg^{-1} soil.

To characterize soils at each site, we analyzed the 2-wk soil sample collection composited for each tree and each soil depth (24 samples). After air-drying and sieving to <2 mm, we determined total carbon and nitrogen by combustion using a Flash EA 1112 (Thermo Scientific), 1 mol L^{-1} NH_4Cl-extractable base cations (Ca, Mg, K, and Na) using a JY Ultima Inductively Coupled Plasma Spectrophotometer (Horiba Jobin Yvon) to determine effective cation exchange capacity (eCEC) and calculated % base saturation, and 0.01 mol L^{-1} $CaCl_2$ soil pH (Brown et al., 2009).

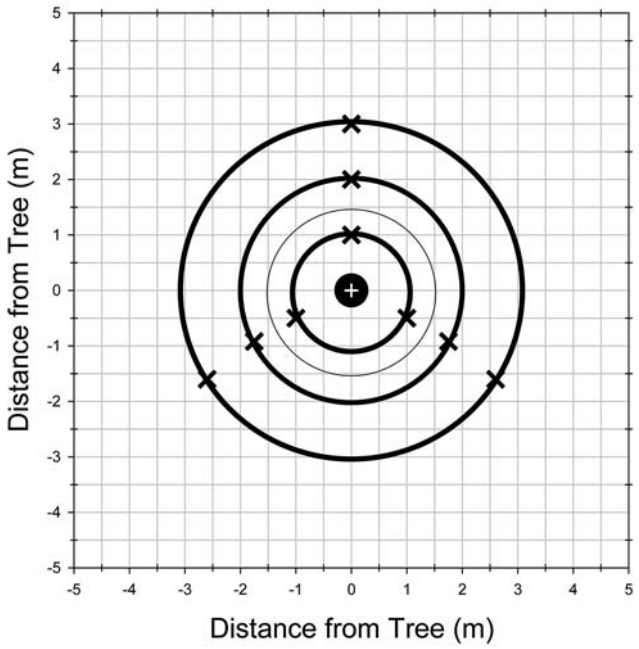

Fig. 1. Diagram showing imidacloprid application circle (thin line), transects with lysimeter locations (X), and soil sample collection distances (thick line) for each tree (*n* = 4) on the high and low elevation sites.

We determined soil physical characteristics, including particle analysis on the <2 mm fraction, bulk density, saturated hydraulic conductivity, and water retention characteristics. Particle analysis was conducted on the composited soil samples described above using the method of Gee and Bauder (1986) as modified by the Coweeta Analytical Lab (Deal et al., 1996). Saturated hydraulic conductivity was determined in situ (after conclusion of the study and removal of the lysimeter network) at a random location 2 m from each treated tree at each site using a Compact Constant Head Permeameter (Amoozemeter, Ksat, Inc.) as described in Amoozegar and Warrick (1986). To examine the water retention characteristics of each site, we collected intact soil cores (4.8 cm diameter by 5 cm long) from each soil layer corresponding to soil collection depths (one core per depth, per tree, per site) of 10 to 15 cm and 30 to 35 cm at both the low- and high-elevation sites plus 70 to 75 cm at the high-elevation site only. We measured water retention using −0.1 and −1.5 MPa ceramic plates (Soil Moisture), for low and high pressure, respectively, in a pressure plate extractor following the method of Klute (1986). The soil cores were used to determine bulk density for each soil layer at each site. After oven drying (105°C), total soil weight and the <2 mm fraction were recorded.

Soil microarthropod populations were surveyed within 1 wk of each soil sample collection for imidacloprid adsorption determinations beneath three treated trees and one reference tree on each site. Three random sample locations were chosen along the 1-m, 2-m, and drip line circular transects for collection; mineral soil was collected in cores 5 cm in diameter and 5 cm deep (9 samples per tree). Microarthropods were immediately extracted from the soil cores using a modified Tullgren funnel apparatus (Mallow and Crossley, 1984). Soil microarthropods were collected in 200-mL vials filled with 70% ethanol and later sorted under a stereo microscope into

the following categories: oribatid, prostigmatid, and mesostigmatid mites; Collembola; and others. Oribatid mites were further sorted into mature and immature categories; mature oribatids have greater sclerotization and thus greater protection from predators (Walter and Proctor, 1999). In addition, the adult and immature forms of some oribatid species feed on entirely different litter (or fungal) material (Schneider et al., 2004). Microarthropod abundances are reported as the mean number of microarthropods per soil core.

Statistics

Differences between high- and low-elevation sites in soil physical and chemical characteristics were determined using ANOVA with the GLM Procedure of SAS (SAS Institute, 2000). Data were analyzed as a split plot with sites representing the whole plot; we used Tree(Site) as the error term. Significant differences among mean values were determined using Tukey adjusted LSMeans. We used the Mixed Procedure of SAS (SAS Institute, 2008) to examine imidacloprid concentrations in soil solution (movement) and soil extractions (adsorbed). Imidacloprid concentration and soil microarthropod data were not normally distributed; we transformed the data (concentration plus one, microarthropod group plus one) using a log-normal distribution. We used Site(Tree) as the covariate term with Repeated time (either week or month). Significant differences ($p \leq 0.10$) among means were determined using Tukey adjusted LSMeans. We analyzed all data together to determine differences between sites and sites alone to examine imidacloprid movement and adsorption. We also determined the significant difference between treated and reference trees using the tree means at each collection date for microarthropod data. We used only data from the treatment hemlock trees to determine differences in imidacloprid response between sites and among distances from the tree. We examined the relationship between microarthropod populations and soil imidacloprid concentrations using the CORR procedure in SAS on log-transformed data (SAS Institute, 2008).

Results

Site and Soil Characteristics

High- and low-elevation sites differed significantly in surface soil temperature and moisture; soils at the high-elevation site were cooler and wetter. There were no significant differences in temperature or moisture among the three distances from the tree. Soil chemical properties differed significantly between the two sites (Table 1). High-elevation soils had greater percent total C and total N in the surface 0- to 20-cm and the 20- to 50-cm depths. Effective cation exchange capacity also differed, with greater eCEC in the high-elevation sites. There were no significant differences in soil pH or % base saturation between the high- and low-elevation sites. Textural analysis categorized soils from both sites, at all depths, as loamy sand, with 75 to 79% sand and 4 to 7% clay (Table 2). Saturated hydraulic conductivity did not differ between sites; however, both locations showed greater conductivity values in surface soils, with the lowest value in the deepest soil layer sampled (30 cm at the low-elevation site and 70 cm at the high-elevation site).

Water retention characteristics differed significantly between sites (Table 2). Total pore space was greater in the high-elevation soils in surface (0–20 cm) and subsurface (20–50 cm) soil. Soil water content at field capacity (−0.03 MPa) was more than two times greater in the high-elevation site compared with the low-elevation site. The 20- to 50-cm soil layer also differed, with water retention at −0.03 MPa being almost three times greater in the high-elevation site. Water content at permanent wilting point (−1.5 MPa) also differed between sites and was greater in both 0- to 20-cm and 20- to 50-cm soils of the high-elevation site compared with the low-elevation site. Hydraulic conductivity did not differ between the high- and low-elevation sites due to high within-site variability. Total bulk density (g cm^{-3}) and density of particles <2 mm (Table 2) were significantly greater in the low-elevation sites.

Imidacloprid Movement in Soil Solution

We examined soil solution imidacloprid concentrations in samples collected during the first 6 wk after imidacloprid injections (Fig. 2) and found no significant differences between sites ($F = 1.07$; $p = 0.34$). Imidacloprid concentrations were greatest closest to the tree (1 m), significantly different from the drip line; concentrations at the 2-m distance did not differ from either 1 m or the drip line. The Site × Depth interaction term was significant ($F = 11.36$; $p = 0.001$), with greater imidacloprid concentrations in soil solutions collected from shallow lysimeters in the high-elevation site compared with the

Table 1. Soil chemical characteristics for high- and low-elevation sites. Values presented are means of soil samples collected from three distances for the four treated trees for each site ($n = 12$).

Depth	C	N	pH	eCEC†	%BS‡
	g kg^{-1}			$cmol_c$ kg^{-1}	
			High elevation		
0–20 cm	81.1§ (5.4)¶	4.4§ (0.02)	4.46 (0.06)	7.21§ (0.43)	34.6 (5.56)
20–50 cm	43.0§ (4.0)	2.1§ (0.2)	4.63 (0.04)	7.93§ (0.44)	32.9§ (3.09)
50–95 cm	23.7 (3.1)	1.2 (0.1)	4.65 (0.06)	6.65 (0.28)	27.3 (1.75)
			Low elevation		
0–20 cm	32.7 (2.1)	2.0 (0.1)	4.37 (0.07)	4.47 (0.18)	44.45 (6.46)
20–50 cm	18.4 (2.3)	1.1 (0.1)	4.64 (0.07)	4.57 (0.28)	47.32 (5.92)

† Effective cation exchange capacity.

‡ Base saturation.

§ Significant difference between the high and low elevation for that depth as determined using the PDIFF option with a TUKEY adjustment in the LSMEANS statement in the GLM procedure of SAS.

¶ Values in parentheses are SEM.

Table 2. Soil physical characteristics for high and low elevation sites. Soil particle analysis values presented are means of soil samples collected from three distances for the four treated trees for each site (n = 12).

Depth	Sand	Clay	*K*sat†	Pore space	FC‡	PWP§	BD¶ (total)	BD (<2 mm)
	—— g kg^{-1} ——		cm h^{-1}	cm^3 cm^{-3}	—— g H_2O g soil^{-1} ——		——— g cm^{-3} ———	
High elevation								
0–20 cm	780 (42)#	40 (7)	18.6 (4.9)	0.71 (0.03)††	0.69 (0.07)††	0.57 (0.06)††	0.69 (0.08)††	0.28 (0.02)††
20–50 cm	780 (59)	60 (15)	23.4 (10.8)	0.68 (0.02)††	0.65 (0.13)††	0.54 (0.11)††	0.74 (0.03)††	0.48 (0.04)††
50–95 cm	750 (68)	70 (15)	8.2 (2.3)	0.63 (0.01)	0.44 (0.08)	0.37 (0.08)	0.92 (0.08)	0.58 (0.07)
Low elevation								
0–20 cm	790 (37)	40 (7)	36.5 (8.6)	0.63 (0.02)	0.32 (0.04)	0.23 (0.03)	1.02 (0.07)	0.61 (0.05)
20–50 cm	790 (57)	60 (9)	8.7 (4.5)	0.51 (0.02)	0.24 (0.04)	0.18 (0.03)	1.29 (0.08)	0.77 (0.04)

† Saturated hydraulic conductivity measured in the field, with four measurements for each site at each soil depth.

‡ Field capacity, representing gravimetric water content at −0.03 MPa.

§ Permanent wilting point (−1.5 MPa). Water potential values were determined on 2.5-cm-diameter by 5-cm-length intact soil cores.

¶ Bulk density determined on 2.5-cm-diameter by 5-cm-length intact soil cores.

Values in parentheses are SEM.

†† Significant difference between the high and low elevation for that depth as determined using the PDIFF option with a TUKEY adjustment in the LSMEANS statement in the GLM procedure of SAS.

low-elevation site. Within a site, there were no significant differences among distance, depth, or w eek. Variability in solution concentrations was v ery high, as noted b y the maximum concentrations measured and the frequent zero values in each collection as shown in Fig. 2. Movement of imidacloprid along preferential flow paths was evident in the high-elevation site in the first soil solution sample collection that occurred 1 wk after application (Fig. 2). Mean soil solution concentration in the deep lysimeter (90 cm below the surface) is 0.7 μg L^{-1}, and the maximum concentration was 6.6 μg L^{-1}.

Analysis of monthly soil solution imidacloprid concentrations found no signifi cant differences between the high- and low-elevation sites or between shallow and deep lysimeter solutions (Fig. 3); however, distance from the tree and imidacloprid injection was signifi cant (F = 7.24; p = 0.001), with gr eater concentrations at the 1 and 2 m distances compar ed with the drip line. There were also significant differences among sample collection months (F = 4.34; p = 0.002) at either the high- or low-elevation sites. S oil solution concentrations in M onth 1 were significantly greater than in Months 3 and 6 but did not differ from Months 9 and 12. Although there were significant

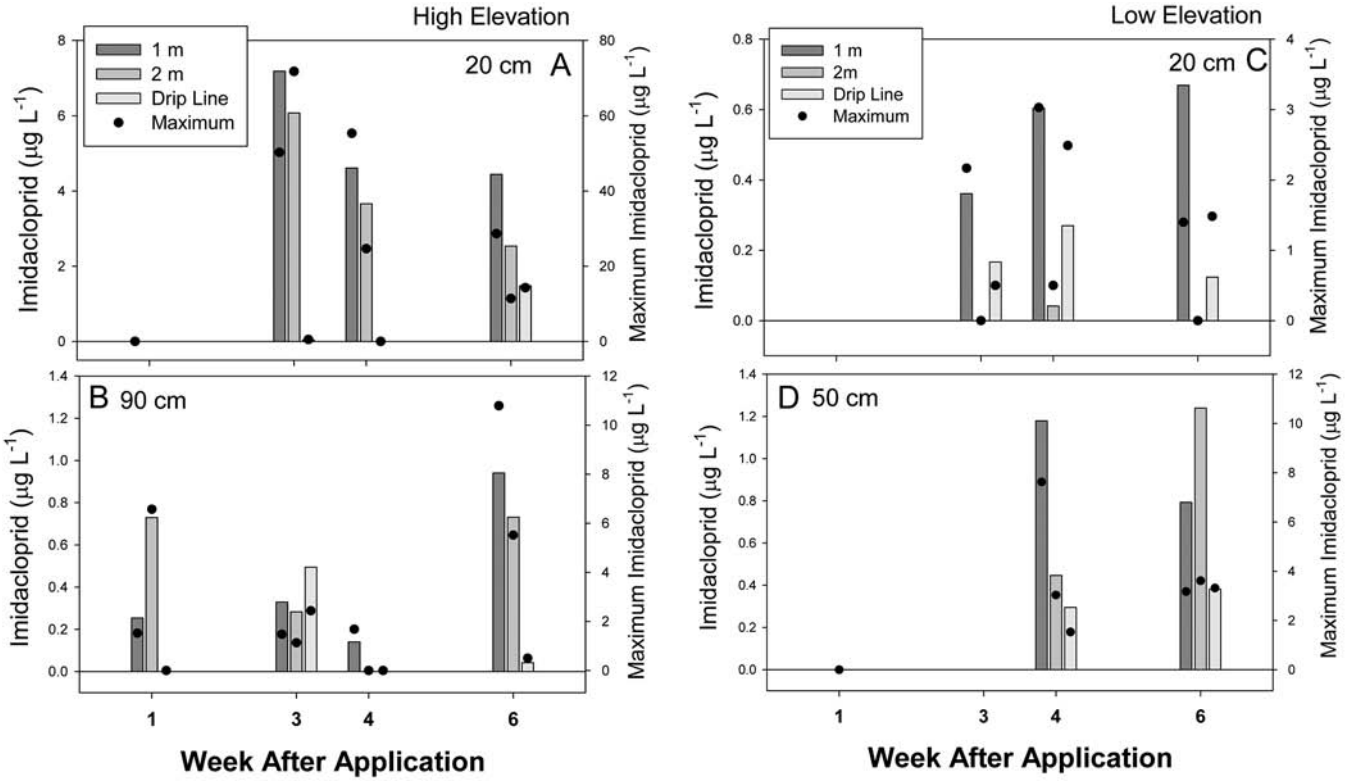

Fig. 2. High (A and B) and low (C and D) elevation soil solution imidacloprid concentrations (μg L^{-1}) for samples collected 1 to 6 wk after soil injection in lysimeters located 1 m, 2 m, and at the tree drip line, 20 cm (A and C) and 50 or 90 cm (B and D) in the soil profile. Bars represent the mean concentrations for each site (four trees per site) each week of collection. Also shown is the maximum solution concentration for each collection week. Note the different scales for mean and maximum concentrations in the 20-cm lysimeter at high- and low-elevation sites.

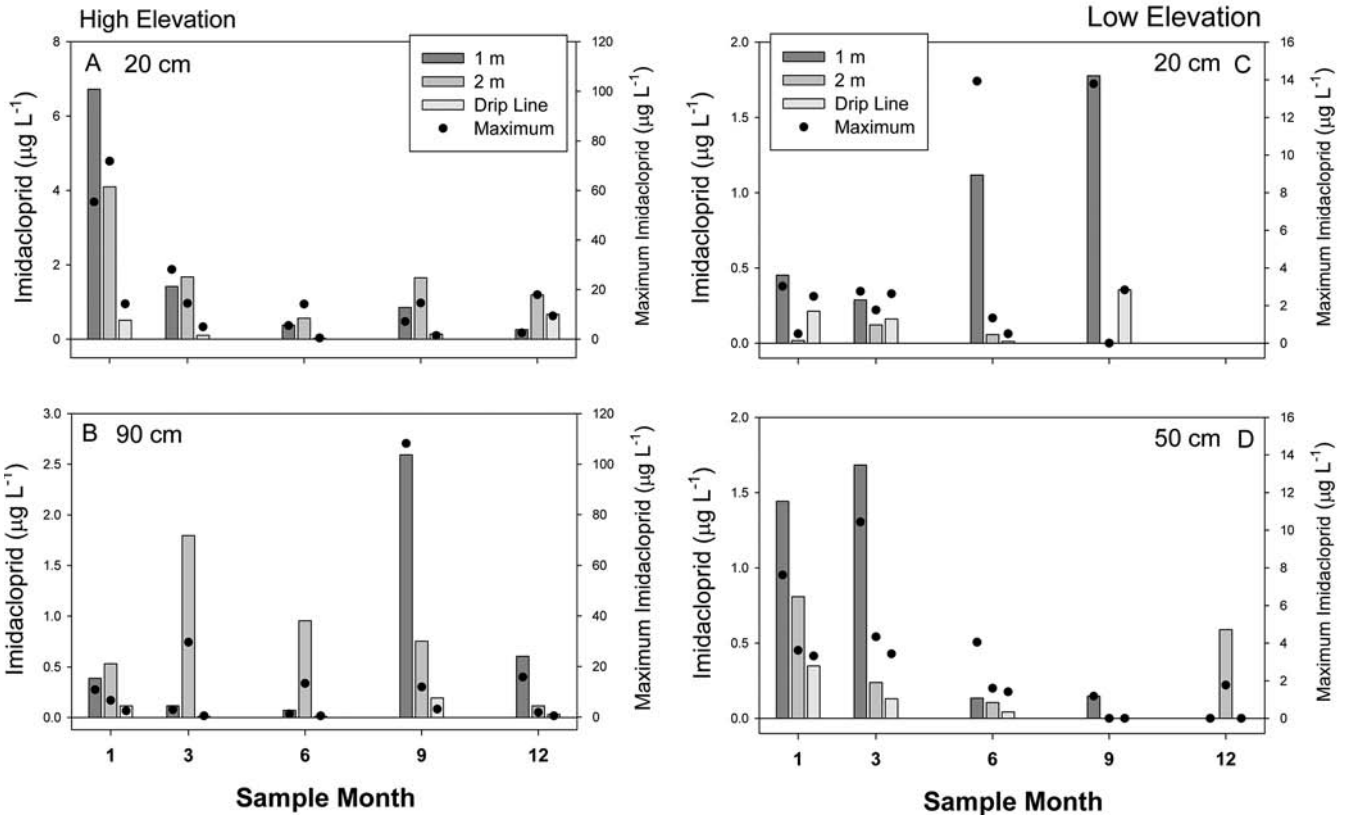

Fig. 3. High (A and B) and low (C and D) elevation soil solution imidacloprid concentrations (µg L^{-1}) for samples collected 1 to 12 mo after soil injection in lysimeters located 1 m, 2 m, and at the tree drip line 20 cm (A and C) and 50 or 90 cm (B and D) in the soil profile. Bars represent the mean concentrations for each site (four trees per site) each week of solution collection. Also shown is the maximum concentration in solution for each collection month. Note the different scales for mean and maximum concentrations in 20 cm and 50 or 90 cm lysimeter at high- and low-elevation sites.

Site × Depth and Site × Depth × Distance interactions, mean separation tests did not identify any differences.

Soil Imidacloprid Concentration

Soil samples were collected 2 wk, 6 mo, and 12 mo after soil injection of imidacloprid to measure extractable soil concentrations (Fig. 4). We propose that imidacloprid present in soil samples collected 2 wk after soil injection moved by preferential flow, whereas subsequent collections represent imidacloprid moving in solution by mass flow. To compare sites for the extent of imidacloprid movement within the soil profile, we used the deepest soil layer sampled, 20 to 50 cm for the low elevation and 50 to 90 cm for the high elevation site. Sites differed significantly in extractable imidacloprid concentration ($F = 21.6$; $p = 0.004$), with concentrations being greater in the high-elevation site (Fig. 4). Distance from the tree was significant ($F = 16.13$; $p < 0.001$), with distances 1 m and 2 m being equal with concentrations of imidacloprid significantly greater than soils collected from the drip line. Due to high variability, there was no significant difference in mean concentrations among depths of sample collection between sites or within a site (Fig. 4). Sample collection dates differed ($F = 13.06$; $p < 0.001$), with imidacloprid concentrations in soil samples collected 2 wk after application being the lowest compared with the 6- and 12-mo collections; the 6-mo collection was also greater than Month 12. Soil imidacloprid concentrations in samples collected 2 wk after injection indicate preferential flow of imidacloprid at the high-elevation site. The deep soil layer (50–90 cm) at 2 m from the tree (0.5 m from the application area) had a mean concentration of 46 µg kg^{-1} with a maximum concentration of 185 µg kg^{-1}, suggesting movement of imidacloprid along a preferential flow path. All other soil imidacloprid concentrations, 2 wk after application, at high- and low-elevation sites, at all soil depths, and at all distances from the tree (and imidacloprid injection site) were below detection limits (<1 µg kg $soil^{-1}$).

Impacts on Soil Microarthropods

High- and low-elevation sites differed significantly in total microarthropod population density but did not differ between treated and reference trees. Populations in the low-elevation site were generally greater for all soil microarthropod groups examined (oribatid mites [$F = 15.21$; $p = 0.02$], prostigmatid mites [$F = 4.3$; $p = 0.11$], and mesostigmatid mites [$F = 20.6$; $p = 0.02$]) as well as Collembola ($F = 5.2$; $p = 0.08$) and total average populations per soil core ($F = 10.9$; $p = 0.03$) (Fig. 5). Populations tended to vary with distance from the tree but not for all groups examined, and samples collected 2 m from the tree had the greatest populations (oribatid [$F = 1.1$; $p = 0.34$], prostigmatid [$F = 2.5$; $p = 0.09$], and mesostigmatid [$F = 1.2$; $p = 0.32$] mites and Collembola [$F = 2.5$; $p = 0.09$] and total populations per soil core [$F = 2.3$; $p = 0.11$]).

Although microarthropod populations did not differ between imidacloprid-treated and untreated reference samples for any group or sample collection month at either the low- or high-elevation site, we did find a significant negative correlation between imidacloprid and microarthropod populations in the low-elevation site. We examined the correlation between

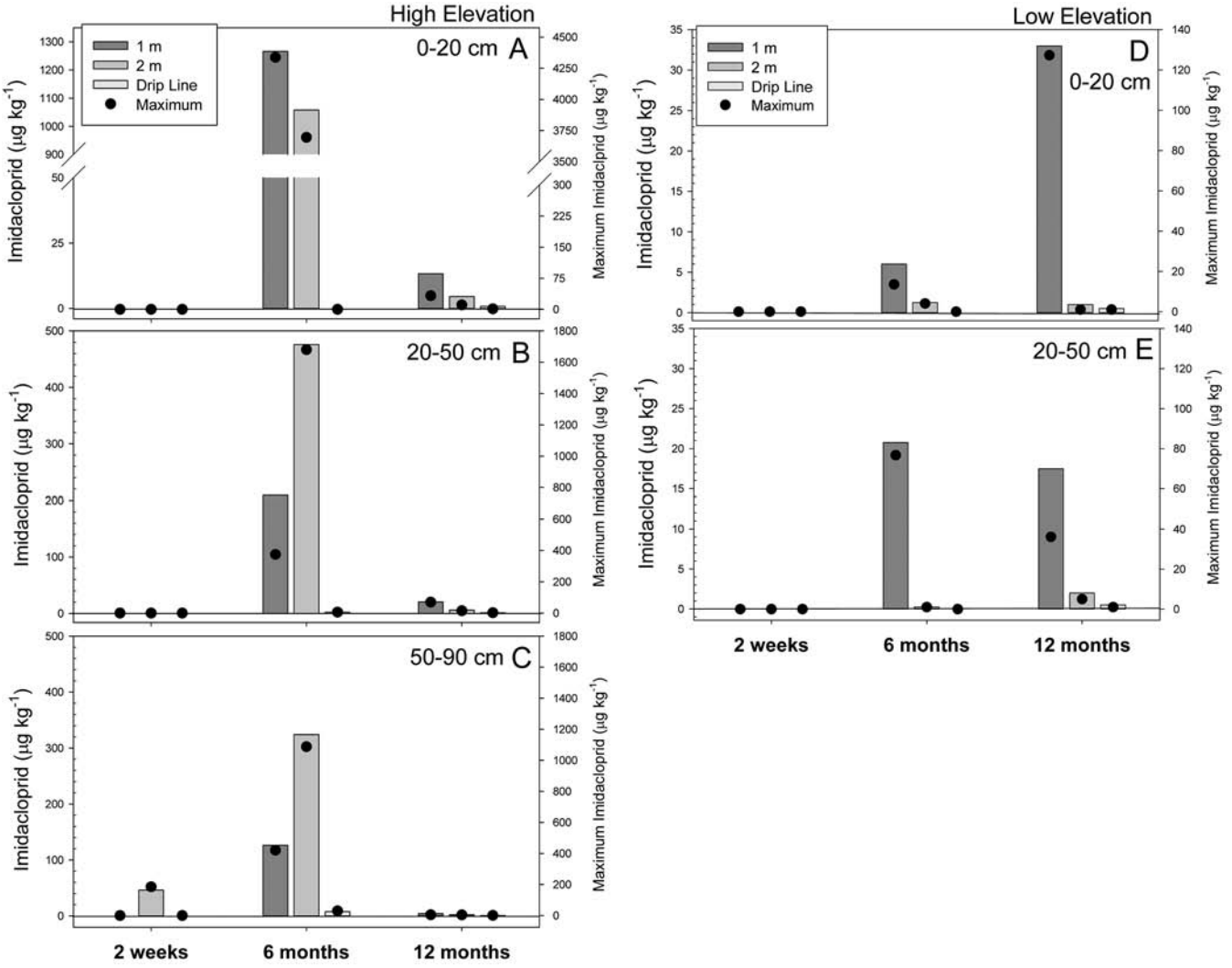

Fig. 4. High (A–C) and low (D and E) elevation acetonitrile-extractable soil imidacloprid concentrations (µg kg^{-1}) for samples collected 2 wk, 6 mo, and 12 mo after soil injection 1 m, 2 m, and at the tree drip line at soil depths of 0 to 20 cm (A and D), 20 to 50 cm (B and E), and 50 to 95 cm (C) in the soil profile. Bars represent the mean concentrations for each site from composite samples collected from four trees per site. Also shown is the maximum soil concentration for each collection month. Note the different scales for mean and maximum concentrations in the 20-cm and 20- to 50-cm soil depths at high and low elevation sites.

microarthropod populations and soil imidacloprid concentrations at all depths using data from the 6- and 12-mo collections (imidacloprid concentrations were zero in the 1-mo sample collection) (Table 3). In the low-elevation site, imidacloprid concentrations at the 20- to 50-cm soil depth were negatively correlated with Collembola, oribatid, and mesostigmatid mites and with the total number of microarthropods per soil core (Table 3). In the high-elevation site, imidacloprid concentrations at 20 to 50 cm were negatively correlated with Collembola only. There were no significant correlations with imidacloprid concentrations in surface soil (0–20 cm) in either site or in the deep soil (50–95 cm) of the high-elevation site. Examining the relationships between soil solution imidacloprid and microarthropod populations showed no significant relationships at either site for shallow or deep lysimeters (data not shown).

Discussion

Soil Adsorption and Movement

Most studies of imidacloprid additions and movement in soils have been conducted in the laboratory using reconstructed soil columns or in soils beneath greenhouses. These studies found that movement of imidacloprid was determined by soil adsorption capacity, which is largely dependent on soil organic matter. However, leaching rates and depth of leachate movement vary greatly among studies due to differences in soil, precipitation, and imidacloprid formulation. Studies examining the relationship between imidacloprid adsorption and movement in soils have examined both soil chemical and physical properties. These studies suggest that clay particle content and soil organic matter are the two main soil characteristics related to imidacloprid adsorption, its subsequent availability to plants, and its movement (Cox et al., 2004; Cox et al., 1998b; Fernandez-Perez et al., 1998; Papiernik et al., 2006). A study by Flores-Cespedes et al. (2002) found that the presence of dissolved organic carbon in soil solution increased imidacloprid mobility and decreased soil adsorption through competition for sorption sites. Although our study sites were initially selected to maximize variability in soil characteristics, the only significant difference between sites was for soil organic

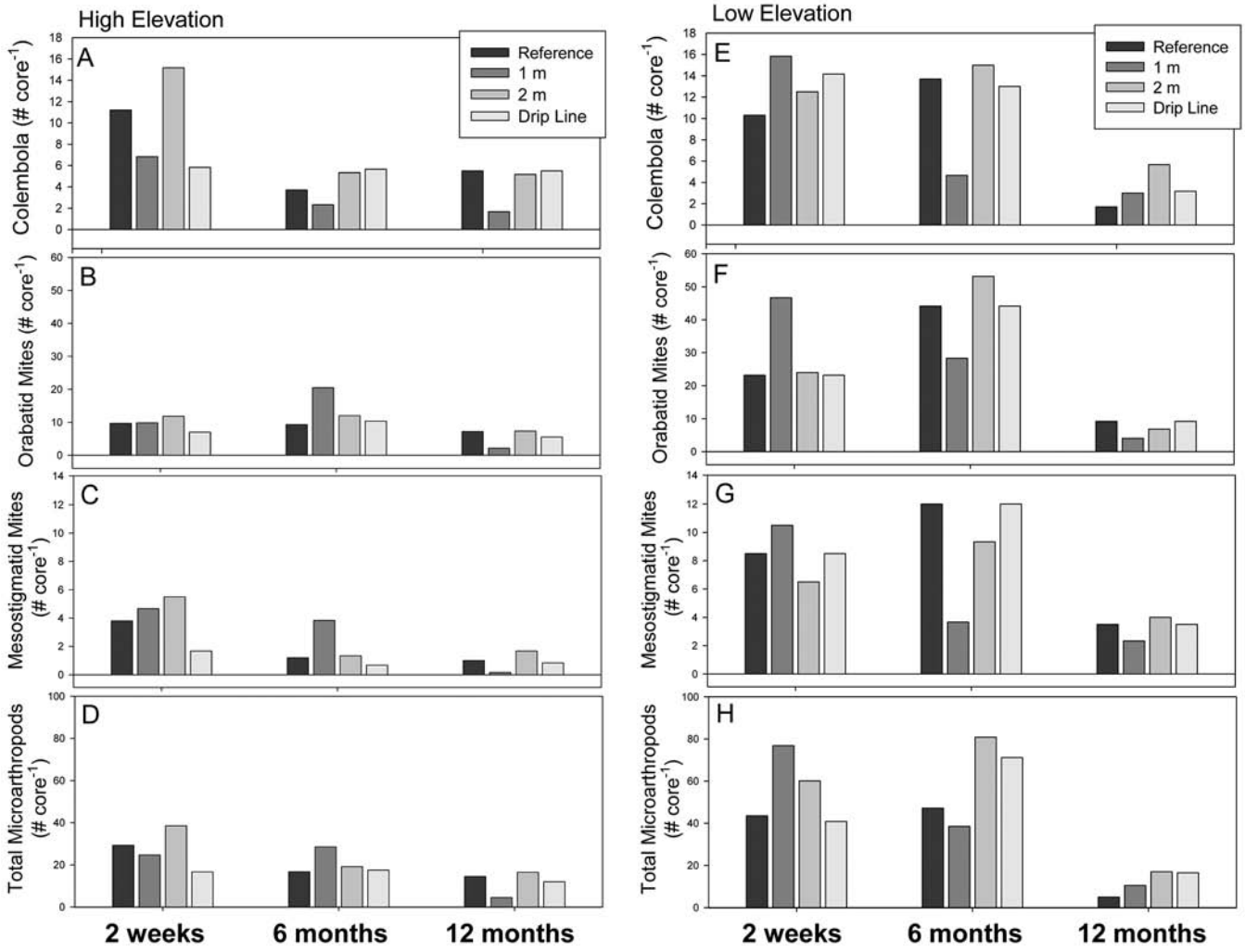

Fig. 5. Microarthropod populations of collembola (A and E), orabatid mites (B and F), mesostigmatid mites (C and G), and total populations (D and H) (number organisms soil core^{-1}) at high-elevation (A–D) and low-elevation (E–H) sites. Core samples were collected 2 wk, 6 mo, and 12 mo after imidacloprid application. Vertical bars represent means for each distance from the tree as well as mean populations at the reference tree for all distances.

matter (i.e., the high-elevation site has more than two times the organic matter content of the low-elevation site) (Table 1).

We found that in soil samples collected 6 and 12 mo after soil injection, extractable imidacloprid concentrations were greatest in the high-elevation site, despite the fact that the high-elevation site receives more than 400 mm greater annual rainfall than the low-elevation site. Gupta et al. (2002) found that more than 60% of the imidacloprid applied was adsorbed by the soil, whereas 29 to 40% moved 25 cm through the soil column in leachate after the addition of 65 cm of water. On average, this is the equivalent of 3 or 4 mo of rainfall at high- and low-elevation sites, respectively, in the southern Appalachians. We attribute differences in soil concentrations between our study sites primarily to the role of organic matter in regulating imidacloprid adsorption and movement. Similarly, Cox et al. (1998a) found that imidacloprid adsorption was maximized about 8 wk after application, largely by soil organic matter. In batch adsorption laboratory studies, Oliver et al. (2005) examined soil characteristics and their role in the adsorption of several pesticides, including imidacloprid, using temperate and tropical soils from Australia and the Philippines. They found that land use (i.e., agriculture versus pasture) had

Table 3. Correlation coefficients for ranked values of Imidacloprid concentration (mg kg^{-1}) and microarthropod populations (number soil core^{-1}).

	Collembola	Oribatid mite	Prostigmatid	Mesostigmatid	Total count
			High elevation		
0–20 cm	NS†	NS	NS	NS	NS
20–50 cm	0.21 (0.01)‡	NS	NS	NS	NS
50–90 cm	NS	NS	NS	NS	NS
			Low elevation		
0–20 cm	NS	NS	NS	NS	NS
20–50 cm	0.20 (<0.01)	0.33 (<0.01)	NS	0.30 (<0.01)	0.33 (<0.01)

† Nonsignificant relationship (regressions with r^2 <0.20 are shown as not significant).

‡ Values in parentheses are probability of significance (*p* value).

a significant impact on adsorption and, within a land use type, on total organic carbon content. Their findings, supported by the findings of Papiernik et al. (2006), suggest that the nature of the organic matter may be more important in its adsorptive capacity than the total organic matter content.

We found that imidacloprid moved over a greater distance than suggested by previous laboratory studies. Felsot et al. (1998) detected imidacloprid up to 105 cm depth in the soil profile, with the greatest imidacloprid concentration at 45 cm depth, and concluded that initial movement of imidacloprid was along preferential flow paths. Examining imidacloprid movement in drained Dutch clay soils, Scorza Júnior et al. (2004) found the greatest concentrations in drainage solution after the first rain event. Similarly, we detected high imidacloprid concentrations in soil solution and soil imidacloprid extractions in deep samples 1 and 2 wk, respectively, after imidacloprid injection 5 cm beneath the mineral soil surface (Fig. 2). This rapid movement is most likely attributable to preferential flow, a phenomenon not likely observed in laboratory studies or in well mixed agricultural soils. Oi (1999) examined imidacloprid adsorption and movement and found that the amount adsorbed increased with time while there was a decrease in soil solution concentrations. We observed a similar temporal pattern in which lysimeter concentrations decline more rapidly than adsorbed soil imidacloprid concentrations.

Concerns about the movement of imidacloprid to streams were addressed by Churchel et al. (2011) in several southern Appalachians streams. They examined imidacloprid concentrations in streams after riparian zone hemlock treatment by stem and soil injection and macroinvertebrate populations in adjacent streams. Only a trace amount of imidacloprid (<1 μg L^{-1} 720 d after application) was detected in one water sample, and no effects on aquatic macroinvertebrates were observed. Negative effects on aquatic organisms have been observed at much greater imidacloprid concentrations. For example, Kreutzweiser et al. (2008a) found reduced survival of stonefly at concentrations of 48 and 96 μg L^{-1}. Tisler et al. (2009) found that of the species they tested most were not very sensitive to imidacloprid; the most sensitive was the water flea, with an EC_{50} for 48 h of 56.6 mg L^{-1}.

Impacts on Soil Microarthropods

Soil microarthropods are of great importance in forest floor and soil organic matter decomposition and nutrient cycling (Coleman et al., 2004); hence, understanding (and limiting) the impacts of imidacloprid on soil microarthropods is critical for maintaining nutrient cycling processes. Some studies have observed little or no effect on soil microarthropods. For example, Peck (2009) and Kunkel et al. (1999) observed no effect of imidacloprid on any *Acari* in studies conducted on turf grass. Elbert et al. (1991) reported no imidacloprid effects on spider mites, whereas other studies have shown pronounced increases in *Tetranychid* populations related to imidacloprid exposure, for which several mechanisms have been proposed (James and Price, 2002). In contrast, Peck (2009) reported reductions in one family of Collembola, the Entomobryomorpha, which he posits may result from their association with the rooting zone, where uptake of imidacloprid occurs. Although we found no difference in total soil microarthropod populations between treated and reference trees, we found weak but significant negative correlations between adsorbed soil imidacloprid and soil microarthropod populations (Table 3) on the treated sites. These results suggest that adsorption of imidacloprid by soil organic matter in southern Appalachian soils may play an important role in localizing, and hence minimizing, the impacts of soil injection applications on soil microarthropod populations.

Our study examined in situ movement and adsorption of imidacloprid after soil injection in two hemlock forest sites. We found that imidacloprid moved to the deepest soil solution lysimeters (90 cm) within 1 wk of application, probably by preferential flow paths. High organic matter content worked to adsorb imidacloprid, making it available to hemlock trees for a longer period of time. At the site with low organic matter, there was a weak but significant negative correlation between soil microarthropod numbers and imidacloprid concentration; however, populations did not differ significantly from untreated reference samples. Because there is considerable variability in riparian zone soils and their organic matter content, it is difficult to make generalizations concerning the movement and adsorption of imidacloprid.

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