Assessment of Imidacloprid and Its Metabolites in Foliage of Eastern Hemlock Multiple Years Following Treatment for Hemlock Woolly Adelgid, Adelges tsugae (Hemiptera: Adelgidae), in Forested Conditions

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ABSTRACT Widespread decline and mortality of eastern hemlock, Tsuga canadensis (L.) Carrière, have been caused by hemlock woolly adelgid, Adelges tsugae (Annand) (HWA) (Hemiptera: Adelgidae). The current study is a retrospective analysis conducted in collaboration with Great Smoky Mountains National Park (GRSM) to determine longevity of imidacloprid and its insecticidal metabolites (imidacloprid olefin, 5-hydroxy, and dihydroxy) in GRSM’s HWA integrated pest management (IPM) program. Foliage samples were collected from three canopy strata of hemlocks that were given imidacloprid basal drench treatments 4–7 yr prior to sampling. Foliage was analyzed to assess concentrations in parts per billion (ppb) of imidacloprid and its metabolites. Imidacloprid and its olefin metabolite were present in most, 95 and 65%, respectively, branchlets 4–7 yr post-treatment, but the 5-hydroxy and dihydroxy metabolites were present in only 1.3 and 11.7%, respectively, of the branchlets. Imidacloprid and olefin concentrations significantly decreased between 4 and 7 yr post-treatment. Concentrations of both imidacloprid and olefin were below the LC50 for HWA 5–7 yr post-treatment. Knowledge of the longevity of imidacloprid treatments and its metabolite olefin can help maximize the use of imidacloprid in HWA IPM programs.

KEY WORDS imidacloprid, olefin, Adelges tsugae, hemlock woolly adelgid, eastern hemlock

Hemlock woolly adelgid, Adelges tsugae (Annand) (HWA) (Hemiptera: Adelgidae), a native of Japan (Havill et al. 2006), has caused widespread mortality in populations of eastern hemlock, Tsuga canadensis (L.) Carrière, and Carolina hemlock, Tsuga caroliniana Engelmann (Pinaeae: Pinaceae). HWA affects hemlocks by depleting carbohydrate storage (Young et al. 1995), reducing photosynthetic ability (Gonda-King et al. 2014, Nelson et al. 2014), reducing growth (Gonda-King et al. 2014), weakening twigs (Soltis et al. 2014), and initiating a hypersensitive response (Radville et al. 2011), eventually leading to tree death.

As a native species, eastern hemlock inhabits a distinctive niche and plays a vital ecological role in southern Appalachian forests as the only shade-tolerant conifer (Orwig and Foster 1998, Ward et al. 2004). Many animal species inhabit eastern hemlock and would be negatively impacted by its decline. For example, eastern hemlock systems have diverse arthropod communities, as more than 400 species of insects and numerous species of spiders are associated with this ecologically important native tree (Wallace and Hain 2000; Buck et al. 2005; Lynch et al. 2006; Dilling et al. 2007, 2009; Hakeem 2008; Mallis and Rieske 2011; Coots et al. 2012).

In the United States, HWA has two generations each year: the sistens generation and the progrediens generation. HWA adults are parthenogenic, and only females are produced by the sistens generation (Young et al. 1995). Sistens hatch in late spring and first-instar nymphs (crawlers) settle on the base of needles, where they remain dormant for several months. They begin to feed and develop during the fall, progressing through several instars. In early spring, sistens produce the progrediens generation eggs. Progrediens crawlers emerge and settle on the base of needles, where they feed for several months. Progrediens produce eggs in late spring, which hatch and begin the sistens generation again (Cheah et al. 2004).

Eastern hemlock has exhibited no widespread resistance against HWA (McClure 1995) and no native predators have sufficiently suppressed HWA populations (McClure 1987). Thus, eastern hemlock is now infested by HWA throughout most of its natural range in the eastern United States (Lambdin et al. 2006). Application of insecticides, particularly imidacloprid,
has been essential in protecting and preserving eastern hemlock in the southern Appalachians.

Imidacloprid, a neonicotinoid insecticide, functions by irreversibly binding to acetylcholine receptors in post-synaptic nerve membranes in the insect central nervous system, causing the eventual termination of nerve impulses (Nauen et al. 2001, Tomizawa and Casida 2005), which can result in insect mortality within 24–48 h (Bai et al. 1991, Mullins and Christie 1995). Once applied to the soil and absorbed by the plant, imidacloprid is metabolized into compounds with insecticidal properties, such as imidacloprid olefin, 5-hydroxy imidacloprid, and 4,5-dihydroxy imidacloprid (henceforth referred to as olefin, 5-hydroxy, and dihydroxy, respectively) (Nauen et al. 1998). Proposed metabolic pathways for imidacloprid indicate hydroxylation of imidacloprid to produce 5-hydroxy, which is then converted to olefin (Nauen et al. 1998, Sur and Stork 2003). The presence of these metabolites, compounded with the effects of imidacloprid, increases the degree of control for this product (Nauen et al. 1998). For example, olefin is about 15 times as toxic as the parent compound to the green peach aphid, Myzus persicae (Sulzer) (Hemiptera: Aphididae), and the cotton aphid, Aphis gossypii Glover (Hemiptera: Aphididae) (Nauen et al. 1998). The persistence and insecticidal properties of these compounds indicate a long-term effect of imidacloprid treatments, beyond the activity of the parent compound alone (Nauen et al. 1998, Cook 2008, Coots 2012). Imidacloprid treatments have shown a high degree of success in reducing populations of HWA (Steward and Horner 1994, Doccola et al. 2003, Webb et al. 2003) for numerous years after application (Cowles et al. 2006, Cook 2008, Dilling et al. 2010, Coots 2012, Coots et al. 2013).

Soil applications of imidacloprid can provide residual insecticidal activity in hemlock trees for numerous years (Cowles et al. 2006, Coots 2012, Coots et al. 2013, Eisenback et al. 2014, Mayfield et al. 2015). This residual activity of imidacloprid in hemlock systems has been attributed to both the long retention of hemlock needles (approximately 3 yr) and the insecticidal capabilities of some of the metabolites of imidacloprid (Nauen et al. 1998, Schönig and Schmuck 2003). After soil applications, imidacloprid can translocate from the roots to the hemlock foliage in effective concentrations for HWA mortality in as little as 3 mo (Tatar et al. 1995, Coots et al. 2013). Imidacloprid concentrations within hemlock foliage peak between 9 and 15 mo after soil application (Dilling et al. 2010, Coots et al. 2013). However, the concentrations of olefin continue to rise for as many as 3 yr after treatment, providing continued HWA suppression (Coots et al. 2013).

The persistence of imidacloprid and olefin in hemlocks is a remarkable phenomenon. As the highest concentrations of imidacloprid are present within 9–15 mo of application followed by reduced concentrations over time (Coots et al. 2013), it is likely that the majority of imidacloprid is absorbed from the soil shortly after application. Residual concentrations may be the result of imidacloprid and olefin, stored within hemlock tissue, continuing to move upwards in the sap over time. Presence of imidacloprid and its metabolites in hemlock over numerous years is likely dependent on initial imidacloprid uptake by hemlocks (Coots et al. 2013), which may be affected by soil moisture conditions near the time of imidacloprid application, soil organic matter content, topography, and hemlock growth rate.

HWA was first documented in the Great Smoky Mountains National Park (GRSM) in 2002 (Lambdin and Grant 2003a, b) and is currently present throughout GRSM wherever hemlocks are located. Eastern hemlock is distributed throughout GRSM, where more than 55,846 ha of hemlock are found. Of that, almost 607 ha are old-growth hemlock and more than 5,665 ha are hemlock-dominated forests (Webster 2010). Changes in hemlock forests owing to HWA damage include a diminished canopy (Orwig et al. 2008), greater light infiltration to the forest floor (Eschtruth et al. 2006), a drier forest floor (Orwig et al. 2008), altered nitrogen cycling in the soil (Jenkins et al. 1999), and more downed woody debris owing to hemlock mortality (Orwig and Foster 1998). GRSM has the potential to suffer the loss of eastern hemlocks and the ecological functions they provide throughout the park owing to HWA-induced mortality.

Once HWA was detected in GRSM, Park personnel began a concerted effort using various chemical treatments (such as insecticidal soap, horticultural oil, imidacloprid, and dinotefuran) and biological control to manage this invasive pest and preserve the hemlock resources. Biological control projects focused primarily on releases of Sasajiscymnus tsugae (Sasaji and McClure) (Coleoptera: Coccinellidae) and Laricobius nigrinus (Fender) (Coleoptera: Derodontidae). Numerous biological control beetle releases have been made in GRSM as part of their integrated pest management program (IPM) (Webster 2010; Hakeem et al. 2010, 2013). Areas for chemical treatment were prioritized based on public access and ecological value. Commonly treated areas included roadways, trails, campgrounds, riparian areas, and conservation areas (Webster 2010). Imidacloprid has been a major component of the HWA management plan in the Park, as more than 225,000 individual trees in GRSM have been treated with imidacloprid.

Since the initiation of their HWA management plan, Park personnel have maintained detailed records on treatments, including treatment dates, dosage rates, and tree measurements. Questions have arisen about how long treatments will be effective, how often treatments should be made, what are the optimal doses for different size hemlocks, is imidacloprid translocated evenly throughout the canopy, and do treatments provide even control throughout the canopy? To address these questions, a multi-year retrospective study was designed to provide information to enhance management of HWA. The objectives of this part of the study were to evaluate the presence, longevity, distribution within the canopy (lower, middle, and upper strata),
and temporal shift in composition of imidacloprid and its metabolites, olefin, 5-hydroxy, and dihydroxy, in hemlocks 4–7 yr after a single imidacloprid treatment. Further analyses for future publications from this retrospective study will explore the concentrations of imidacloprid and its metabolites in hemlocks in differing diameter size classes, as well as population suppression of HWA and hemlock health numerous years after imidacloprid treatment.

Materials and Methods

Site/Tree Selection. Previous studies have focused on hemlock trees of similar sizes. However, this retrospective analysis aims to assess the longevity of imidacloprid treatments from a range of hemlock sizes found in a typical forest management program to better inform comprehensive management decisions. Potential study sites were selected based on information in the GRSM hemlock treatment database, discussions with Park personnel, and site visits. To isolate the effects of single imidacloprid treatments, sites where hemlocks were treated once with imidacloprid were of interest. Owing to sites receiving multiple treatments or treatments with additional pesticides, site availability in GRSM was limited. Final site selections were based on treatment method, time of treatment, geographic location, elevation, and hemlock forest composition (hemlock dominant or co-dominant). Sites where hemlocks received imidacloprid treatments 4–7 yr before this project was initiated were selected. To increase the likelihood that sites experienced similar environmental conditions, geographic proximity and similar elevations were also important factors in site selection. The elevation (10-m resolution) of each hemlock tree was determined using ArcMap 10 (Environmental Systems Resource Institute [ESRI] 2010). Because a drought occurred during the summer of 2007, and to ensure that all study trees experienced similar stressors, sites that had been treated with imidacloprid prior to the drought were chosen for this study.

Eastern hemlock trees \( (n = 102) \) were selected at two sites (Anthony Creek \( 35° 35.682 \text{ N}, –83° 45.845 \text{ W} \) and Hesse Creek \( 35° 40.190 \text{ N}, –83° 52.126 \text{ W} \)) on the western side of GRSM and one site on private land (Mountain Homes, Inc. \( 35° 40.574 \text{ N}, –83° 52.144 \text{ W} \)) adjacent to the Park (Table 1; Fig. 1). Hemlocks at each site were sampled in 2012 and 2013. Imidacloprid treatments were applied at Mountain Homes \( (n = 33 \text{ trees}, 17 \text{ ha}, \text{ elevation: } 350 \text{ m}) \) in December 2007, 4 yr before initiation of sampling in January 2012 (Table 1). Treatments at Hesse Creek \( (n = 35 \text{ trees}, 6 \text{ ha}, \text{ elevation: } 311 \text{ m}) \) were applied in 2006, 5 yr before sampling began. Hemlocks at Hesse Creek and Mountain Homes, Inc., would have had effective concentrations of imidacloprid in the spring following treatment. Trees at Anthony Creek \( (n = 34 \text{ trees}, 21 \text{ ha}, \text{ elevation: } 671 \text{ m}) \) received imidacloprid treatments in 2006, 5 and 1-half yr before sampling began in 2012 (Table 1). Treatments were applied to eastern hemlock at Anthony Creek during the late summer and early fall of 2006. As imidacloprid can reach effective concentrations in the canopy at 3 no post-treatment (Tattar et al. 1998, Coots et al. 2013), the Anthony Creek site will be classified as 6 yr post-treatment in 2012 and 7 yr post-treatment in 2013 throughout the remainder of this paper. At each site, hemlocks ranged from 30.5 to 76.2 cm in diameter at breast height (DBH) and ranged from 18.3 to 36.6 m in height. The DBH of hemlocks were evenly distributed among sites. Trees were marked with metal identification tags, as well as flagging tape, to enhance identification of study trees. As the year post-treatment ranged from 4 to 6 yr in 2012 and 5 to 7 yr in 2013, more trees were sampled 5 and 6 yr post-treatment as compared with 4 and 7 yr post-treatment because of the overlap in year post-treatment between 2012 and 2013.

Insecticide Application. Hemlocks were treated with a basal drench of imidacloprid (material is poured into the soil within 0.6 m of the base of the hemlock trunk) during 2006 and 2007 (Table 1). Imidacloprid dosage varied among sites and hemlock sizes. All hemlocks at Anthony Creek received a low-dose imidacloprid treatment \( (0.7 \text{ g active ingredient [AI]}/2.5 \text{ cm DBH}) \). At Hesse Creek and Mountain Homes, hemlocks less than 63.5 cm DBH were given the same low-dose imidacloprid treatment \( (0.7 \text{ g AI}/2.5 \text{ cm DBH}) \), while trees 63.5 cm DBH and greater were given a high-dose treatment \( (1.4 \text{ g AI}/2.5 \text{ cm DBH}) \). This dosage increase on larger trees at Hesse Creek and Mountain Homes was part of the HWA management program at both locations (Jesse Webster, personal communication).

Foliage Sampling and Canopy Stratification. Three branchlet samples \( (0.5\text{-m-long}) \) were randomly collected from each of three strata (lower third, middle third, and upper third) of the live canopy of each hemlock selected at each site during

<table>
<thead>
<tr>
<th>Site</th>
<th>Elevation (m)(^a)</th>
<th>Slope (degrees)</th>
<th>Aspect (degrees)</th>
<th>No. trees sampled per site</th>
<th>Treatment date(^b)</th>
<th>Sampling date(^c)</th>
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<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2006</td>
<td>7 Feb.</td>
</tr>
</tbody>
</table>

\(^{a}\) Average elevation in meters at each site.  
\(^{b}\) Date imidacloprid was applied to soil underneath hemlock trees.  
\(^{c}\) Date branchlet samples were collected for analysis.
the winters of 2012 and 2013 (Table 1) to assess the translocation of imidacloprid and its insecticidal metabolites, olefin, 5-hydroxy, and dihydroxy, within the canopy. The amount of live canopy varied widely among trees, depending on height, dominance/codominance, etc. For example, heights from the ground surface to the bottom of the live canopy ranged from approximately 1.5 to 30.0 m, with some of the large trees (i.e., 30.0 m) having a small live crown ratio (i.e., 30%). Owing to the height of the hemlocks and the remote location of the sites, tree climbers (Appalachian Arbors and GRSM personnel) ascended the trees with pre-labeled plastic bags (7.5-liter) and collected foliage samples using pole pruners. However, some lower-stratum branchlet samples were collected with hand pruners, where possible. Branchlets were randomly selected for collection in each stratum of the tree. As samples were collected, they were placed into plastic bags and lowered to the ground in cloth buckets (approximately 19 liters). All samples from each tree were placed in plastic bags (49 liter) for transport to the laboratory. Once in the laboratory, samples were placed in a walk-in cooler (4°C) for as many as 5 d to prevent mold growth on the foliage until sample preparation.

Sample Preparation. Branchlets were removed from the cooler and clipped into smaller sections (approximately 25 cm) within 5 d of arrival to the laboratory, and branchlet samples were placed in labeled brown paper bags (2.900 cm³) for drying. Samples were air-dried at 21°C until needles easily detached from the twigs (approximately 2 wk). Dried needle tissue was pulverized using a Mr. Coffee™ coffee grinder (IDS77-NP, Rye, NY) within 3 mo of needle detachment. Processed samples were then placed in centrifuge tubes (50 ml) and stored in a dark, dry location. Pulverized needles (approximately 1 g) were placed in centrifuge tubes (15 ml) (Fischer Scientific, USA) and 10 ml of acetonitrile was added. A 1:10 (needle:solvent) ratio was used to extract the compounds from the hemlock needles, as this ratio is adequate for needle extraction (Cowles et al. 2006). The centrifuge tube was shaken overnight on an orbital bench shaker (New Brunswick Scientific, Edison, NJ; model G33). Approximately 200 μl of supernatant acetonitrile was then passed directly through a 0.2-μm nylon filter into an autosampler vial containing a 300-μl vial insert for liquid chromatography tandem mass spectrometry (LC/MS/MS) analysis. Spike recovery experiments provided sufficient recoveries (91–102%) for the target compounds of interest (Cook 2008).

Source of Chemicals. Imidacloprid was purchased from Supelco and the imidacloprid metabolites (olefin, 5-hydroxy, and dihydroxy) were provided by Bayer Agrochemical. These chemicals were used as standards.
in chemical analysis of hemlock foliar samples. Acetonitrile was HPLC grade (Fisher Scientific). All chemicals were used as received.

**LC/MS/MS.** Imidacloprid and its metabolites were quantified at Villanova University by LC/MS/MS. A Shimadzu Prominence HPLC system consisting of binary Shimadzu LC-20AD pumps and SIL-20A autosampler (Shimadzu, Colombia, MD) was used under Analyst software control (Applied BioSystems/SCIEX, Framingham, MA) for HPLC separation. A Phenomenex Gemini NX (C18, 4.6 by 250 mm, 5-μm particle) fitted with a 2-mm guard column was used for separation. An aqueous (water with 10 mM ammonium formate) phase and an organic (acetonitrile) mobile phase were used at a total flow of 1.0 mL·min⁻¹. A gradient programmed elution was used that ramped from 25 to 95% acetonitrile (1-8 min), used a column wash with 95% acetonitrile (8-9.5 min), and had a column stabilization period with 25% methanol prior to the next injection. A 10 μL injection volume was used for imidacloprid and metabolite standards and the samples that were analyzed by LC/MS/MS.

Mass spectroscopy was performed with an Applied BioSystems/SCIEX 3200 Q-TRAP triple quadrupole mass spectrometer (Framingham, MA) operated in positive electrospray ionization (ESI) mode. Multiple reaction monitoring (MRM) transitions were optimized using standards (Table 2) (Cook 2008). Optimized ESI source parameters were as follows: curtain gas (CUR) = 35 psi, CAD gas = medium, ESI nebulizer gas (GAS1) = 60 psi, auxiliary gas (GAS2) = 60 psi, ESI probe temperature = 550°C, and ion spray voltage (IS) = 5500 V. The collision exit potential (CXP) was maintained at 4 V and the Q0 entrance potential (EP) was maintained at 10 V for all compounds. The resolution of Q1 and Q3 was fixed at high resolution and the dwell time for each MRM transition was 500 ms.

Analytical MS precursor and fragment ions and their optimized voltages as well as the analytical sensitivity of the LC/MS/MS method are summarized in Table 2. Standards for each compound were analyzed in the ranges provided. For each compound, the concentration (ppb) limit of detection (LOD) is calculated at a signal-to-noise ratio of 3 based on a low-concentration standard (Table 2). This concentration LOD is converted to an on-column LOD using the injected volume (10 μL for all compounds).

**Data Analysis.** All data were entered in an Excel file (Microsoft, Redmond, WA). Data points below the LOD for each chemical were given a zero value. The LOD for olefin is 1.05 ± 0.32 ppb, and the LC₅₀ of olefin for HWA is 6 ppb, determined in a laboratory study through 15-d exposure duration (Coots 2012). Using the LOD rather than zero could artificially inflate averages relative to this LC₅₀. Because management recommendations will be made from the analysis of these data, the most conservative approach was used. Chemical concentration data were tested for normality using Shapiro–Wilks and Kolmogorov–Smirnov tests. Owing to the wide range of imidacloprid and olefin concentrations, log transformations (ln(x+0.1)) were used on these data prior to analysis. Boxplots were generated for both imidacloprid and olefin concentrations for years post-treatment to illustrate the wide range of data distribution. Mixed-model ANOVA and least significant difference (LSD) mean separation procedure were conducted on imidacloprid and olefin concentration data (P < 0.05) using the GLIMMIX procedure in SAS (SAS Institute Inc. 2008). This analysis was conducted to determine differences between sampling years (2012 and 2013) and among sampling sites (Anthony Creek, Hesse Creek, and Mountain Homes) with tree by sampling year by site as a random effect. ANOVA was conducted to determine differences among years post-treatment (4, 5, 6, and 7) and canopy strata (lower, middle, and upper) with sampling year and sampling year by tree by years post-treatment as random effects. All means presented in results are back-transformed from the means of log-transformed data used in analyses.

### Results and Discussion

**Presence and Distribution of Imidacloprid and Its Metabolites.** Imidacloprid and its metabolites were recovered from hemlock foliage for as many as 7 yr after treatment. The majority of branchlets sampled during 2012 and 2013 contained imidacloprid (95.0%) and olefin (65.5%) concentrations above their LOD. However, few branchlets had detectable concentrations of 5-hydroxy (1.3%) and dihydroxy (11.7%). Thus, the 5-hydroxy and dihydroxy metabolites 4–7 yr post-treatment are unlikely contributors to HWA suppression, and were not included in data analyses. The remainder of this paper focuses on imidacloprid and olefin, as these chemicals are present in most of the branchlets sampled.

A wide range of imidacloprid (Fig. 2) and olefin (Fig. 3) concentrations (0 to 2,371 ppb and 0 to 1,134 ppb, respectively) was observed. Concentrations were more widely distributed for both imidacloprid
and olefin in the 4 yr post-treatment group compared with the 5, 6, and 7 yr post-treatment groups. Some branchlets had high concentrations of imidacloprid and olefin in excess of 500 ppb. Similarly, high concentrations of imidacloprid have been observed by Cook (2008) and Eisenback et al. (2010). Forty branchlets had concentrations of imidacloprid exceeding 500 ppb, and 82% occurred in trees 4 yr post-treatment. In addition, 10 of the 13 branchlets with olefin concentrations in excess of 500 ppb were from samples collected 4 yr post-treatment. The branchlets with high olefin concentrations generally had high imidacloprid concentrations. Twelve of the 13 branches with olefin concentrations above 500 ppb have concentrations of imidacloprid in excess of 500 ppb, while all branchlets with imidacloprid concentrations in excess of 500 ppb contained olefin concentrations greater than 150 ppb.

Fig. 2. Distribution of imidacloprid concentration data 4–7 yr after basal drench treatments. Boxes contain the 25th to the 75th percentiles of the data. End points of the whiskers indicate the 10th and 90th percentiles of the data. Circles indicate the 5th and 95th percentiles of the data. The median is indicated by a solid line in each box. The dashed lines indicate the arithmetic mean.

Fig. 3. Distribution of olefin concentration data 4–7 yr after basal drench treatments. Boxes contain the 25th to the 75th percentiles of the data. End points of the whiskers indicate the 10th and 90th percentiles of the data. Circles indicate the 5th and 95th percentiles of the data. The median is indicated by a solid line in each box. The dashed lines indicate the arithmetic mean.
Imidacloprid Concentration – Site and Sampling Year. Imidacloprid concentrations in branchlets were significantly different between 2012 and 2013 ($F = 121.99; \text{df} = 1, 199; P < 0.001$) (data not shown). Concentrations of imidacloprid were significantly higher in 2012 compared with those in 2013 ($P < 0.05$, LSD test). There were site-specific differences in concentration of imidacloprid (data for sampling years combined) ($F = 8.78; \text{df} = 2, 199; P < 0.001$) as well as site by sampling year interaction for imidacloprid concentrations ($F = 8.99; \text{df} = 2, 199; P < 0.001$). While each site had a higher concentration of imidacloprid in 2012 compared with 2013, the concentration of imidacloprid in foliage collected from hemlock at Mountain Homes was significantly higher than imidacloprid concentrations at all other sites in 2012 but significantly lower than Hesse Creek in 2013 ($P < 0.05$, LSD test) (Fig. 4A). Imidacloprid concentrations at Anthony Creek in 2013 were not significantly different than those at Mountain Homes, but were significantly lower than those at Hesse Creek ($P < 0.05$, LSD test). The significant reduction in imidacloprid concentrations in foliage at Mountain Homes from 2012 to 2013 may be influenced, in part, owing to a small number of branchlets with high concentrations of imidacloprid in 2012.

![Imidacloprid Concentration](image)

**Fig. 4.** Comparison of imidacloprid (A) and olefin (B) concentrations in hemlock foliage in 2012 and 2013. Means displayed are back-transformed from log-transformed means used in the statistical analysis. Bars on each column denote standard error of the mean. Columns with the same letters are not significantly different ($P < 0.05$; LSD test). An interaction effect exists between the sites and the year of sampling for imidacloprid concentrations (A). Olefin concentrations had no interaction effect among the sites and between the year of sampling (B).
Dose–response assays have documented the LC$_{50}$ of imidacloprid for HWA as 112 and 300 ppb (Cowles et al. 2006, Coots 2012), from laboratory experiments that by necessity involved exposure of 15 d and 20 d, respectively. In forest settings, suppression of the HWA progrediens generation has been observed when imidacloprid concentrations are greater than 120 ppb 2 yr post-treatment (Cowles et al. 2006). In the current study, the highest average imidacloprid concentration per site and year post-treatment documented was 69.4 ppb from Mountain Homes in 2012, 4 yr post-treatment. The average imidacloprid concentrations at all sites during 2012 and 2013 were below the 15 and 20 d LC$_{50}$ for HWA, so while the imidacloprid that is present may contribute to HWA population suppression, the concentration of imidacloprid in foliage tissue appears insufficient to cause HWA mortality.

**Olefin Concentration – Site and Sampling Year.** Overall average olefin concentration in 2012 (12.8 ± 2.3 ppb) was significantly different from the average olefin concentration in 2013 (0.88 ± 0.17 ppb) ($F_{1, 5} = 108.24, df = 1, 199; P < 0.001$). Concentrations in 2012 were 14× higher than concentrations in 2013. Olefin concentrations in foliage at each site were higher in 2012 compared with 2013 ($P < 0.05$, LSD test) (Fig. 4B). Olefin concentrations differed among sites ($F_{5, 199} = 5.13; df = 2, 199; P < 0.007$) (Fig. 4B), and no significant interaction between site and sampling year was detected ($F = 0.11; df = 2, 199; P = 0.795$). As an interaction effect was not detected, differences among sites are based on olefin concentrations from 2012 and 2013 combined. Hesse Creek and Mountain Homes, the more recently treated sites, had higher concentrations of olefin than Anthony Creek ($P < 0.05$, LSD test).

More branchlets with high olefin concentrations and detectable concentrations of olefin were present in 2012 compared with 2013. While some branchlets sampled in 2013 had higher olefin concentrations, that occurrence was not observed to the degree seen in 2012. In addition, reductions in the percentage of branchlets with concentrations above the LOD for olefin were observed between 2012 and 2013. In 2012, 83.5% of branchlets contained olefin concentrations above the LOD, while only 46.4% did in 2013. The observed reduction of branchlets with high olefin concentrations and a reduction in the number of branchlets with olefin at detectable concentrations explain the lower concentration found in 2013.

Olefin concentrations from all sites in 2012 exceeded the 15-d LC$_{50}$ for HWA. The 15-d LC$_{50}$ and LC$_{50}$ of olefin for HWA are 6 and 7 ppb, respectively (Coots 2012). Olefin concentrations at all sites in 2012 were above 6 ppb, so sufficient levels of olefin were present at all sites in 2012 to contribute to moderate-to-high levels of HWA mortality. Hemlocks from Hesse Creek and Mountain Homes experienced olefin concentrations above the 15-d LC$_{50}$ in 2012. However, the average olefin concentration from samples in 2013 was lower than those in 2012. The highest average olefin concentration observed in 2013 was only 1.2 ppb from Mountain Homes. The concentration of olefin at each site in 2013 was below the 15-d LC$_{50}$ for HWA.

### Table 3. Comparison of imidacloprid and olefin concentrations in foliage 4–7 yr after imidacloprid treatment

<table>
<thead>
<tr>
<th>Years post-treatment</th>
<th>n</th>
<th>Imidacloprid concentration (ppb)</th>
<th>Olefin concentration (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>33</td>
<td>41.05 ± 23.33a</td>
<td>6.36 ± 7.23a</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>18.31 ± 10.03b</td>
<td>4.61 ± 5.12a</td>
</tr>
<tr>
<td>6</td>
<td>69</td>
<td>25.75 ± 14.08b</td>
<td>2.76 ± 3.11b</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>9.74 ± 5.75c</td>
<td>1.65 ± 1.96c</td>
</tr>
</tbody>
</table>

Means (±SE) within a column followed by the same letters are not significantly different ($P > 0.05$, LSD test). Means displayed are back-transformed from log transformed means used in the statistical analysis. Significance is determined by standard error of the difference between means.

### Table 4. Comparison of imidacloprid and olefin concentrations in foliage in each canopy stratum

<table>
<thead>
<tr>
<th>Canopy stratum</th>
<th>Imidacloprid concentration (ppb)</th>
<th>Olefin concentration (ppb)</th>
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<tbody>
<tr>
<td>Lower</td>
<td>20.02 ± 10.81b</td>
<td>3.37 ± 3.73a</td>
</tr>
<tr>
<td>Middle</td>
<td>24.55 ± 13.27a</td>
<td>3.77 ± 4.16a</td>
</tr>
<tr>
<td>Upper</td>
<td>18.42 ± 9.96b</td>
<td>3.13 ± 3.47a</td>
</tr>
</tbody>
</table>

Means (±SE) within a column followed by the same letters are not significantly different ($P > 0.05$, LSD test). Means displayed are back-transformed from log transformed means used in the statistical analysis. Significance is determined by standard error of the difference between means.

### Imidacloprid Concentration – Years Post-Treatment and Canopy Strata.

Significant differences in average imidacloprid concentrations among years post-treatment were detected ($F = 8.60; df = 3, 201; P < 0.001$). Imidacloprid concentrations 4 yr post-treatment were significantly higher than imidacloprid concentrations 5, 6, and 7 yr post-treatment ($P < 0.05$, LSD test) (Table 3). High concentrations in relatively few branchlets collected 4 yr post-treatment may have influenced these results. Concentrations of imidacloprid 7 yr post-treatment were significantly lower than average imidacloprid concentrations 4, 5, and 6 yr post-treatment ($P < 0.05$, LSD test). No significant difference between imidacloprid concentrations 5 and 6 yr post-treatment was detected ($P > 0.05$, LSD test). The concentrations of imidacloprid in foliage decreased from 4 to 7 yr post-treatment, so that as time since treatment increases, the concentration of imidacloprid decreases. Similar observations of decreases in imidacloprid concentrations at sites over time have been observed by Coots et al. (2013) where imidacloprid peaked at approximately 1 yr after treatment, and then experienced a steady decline until 3 yr post-treatment when the study ended. In addition, imidacloprid concentrations are below the 15- and 20-d LC$_{50}$ for HWA 4–7 yr post-treatment.

Significant differences in imidacloprid concentrations among hemlock canopy strata, data combined for all years post-treatment ($F = 9.02; df = 2, 1632; P < 0.001$), were detected (Table 4). The middle stratum of the hemlock canopy exhibited the highest concentration of imidacloprid ($P < 0.05$; LSD test).
Concentrations of imidacloprid were not significantly different in the lower and upper strata ($P > 0.05$; LSD test). No significant interaction effect between years post-treatment and canopy strata was observed (Table 5). While this study found higher concentrations of imidacloprid in the middle stratum of the hemlock canopy, Coots et al. (2013) observed higher concentrations of imidacloprid in the lower stratum of hemlocks. The differences in levels of imidacloprid among canopy strata could be owing to site-specific parameters or the size of hemlocks sampled in the studies. The hemlocks sampled in Coots et al. (2013) were 23.5 to 29.5 cm DBH, while hemlocks sampled in this study were 30.5 to 76.2 cm DBH. Thus, tree size may be a factor in the translocation of imidacloprid throughout the canopy.

### Olefin Concentrations – Years Post-Treatment and Canopy Strata

Olefin concentration decreased as years post-treatment increased ($F = 2.91$; $df = 3, 201$; $P = 0.036$) (Table 5), and no significant difference in olefin concentrations among canopy strata was detected ($F = 1.53$; $df = 2, 1632$; $P = 0.218$) (Table 4). However, significant interactions between years post-treatment and canopy strata for olefin concentrations were detected ($F = 2.39$; $df = 6, 1632$; $P = 0.026$). Olefin concentrations among canopy strata or years post-treatment in samples collected 4 and 5 yr post-treatment were not significantly different ($P > 0.05$; LSD test) (Table 5). Olefin concentrations in the lower and middle strata of the canopy 6 and 7 yr post-treatment were not significantly different ($P > 0.05$; LSD test); however, the upper stratum had significantly lower concentrations than the lower and middle strata 6 yr post-treatment ($P < 0.05$; LSD test). The upper stratum 6 yr post-treatment and the lower and upper strata 7 yr post-treatment had significantly lower olefin concentrations than 4 and 5 yr post-treatment ($P < 0.05$). Distribution of olefin in the canopy is similar among strata in more recently treated trees. Hemlocks 6 and 7 yr post-treatment experienced lower concentrations of olefin in the upper strata. However, higher concentrations of olefin in the upper and middle strata compared with the lower stratum have been observed (Coots et al. 2013). Overall trends in the data indicate lower olefin concentrations as years since imidacloprid treatment increase.

Concentrations of olefin exceeded the olefin 15-d LC$_{50}$ (6 ppb) for HWA in the upper stratum 4 yr post-treatment, and all other strata 4–7 yr post-treatment experienced less than 6 ppb of olefin in the foliage. Concentrations of both imidacloprid and olefin in most of the canopy strata 4–7 yr post-treatment are below the 20-d and 15-d, respectively, LC$_{50}$ for HWA.

In conclusion, imidacloprid and three of its metabolites (olefin, 5-hydroxy, and dihydroxy) were present in hemlock foliage collected from trees 4–7 yr after those trees received one imidacloprid basal drench treatment. A low percentage of branchlets contained 5-hydroxy and dihydroxy (1.3% and 11.7%, respectively), and the metabolites are not considered to be a contributing factor to effective chemical suppression of HWA 4–7 yr post-treatment. A wide range of imidacloprid and olefin concentrations was documented in foliage, especially 4 yr post-treatment. Average imidacloprid concentrations were below the 15- and 20-d LC$_{50}$ for HWA 4–7 yr post-treatment. Average olefin concentrations were above the 15-d LC$_{50}$ for HWA 4 yr post-treatment. Over time, the concentrations of imidacloprid and olefin decreased. Concentrations in 2013 were lower than those in 2012, and this reduction was observed at each site. Higher concentrations of imidacloprid were present in the middle stratum of the hemlock canopy. While olefin concentrations among strata varied 6 and 7 yr post-treatment, no differences in olefin concentrations among canopy strata 4 and 5 yr post-treatment were detected.

Knowledge of the persistence of imidacloprid and olefin and their possible combined additive effect can be used to extend treatment efficacy for longer periods and facilitate better use of imidacloprid treatments in HWA IPM programs. Treating hemlock trees less often offers HWA management programs both financial and environmental benefits. Financial resources can be saved by treating trees less often, while adding imidacloprid to the forest system less frequently reduces the risk of potential nontarget impacts.

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