

# Impact of Imidacloprid for Control of Hemlock Woolly Adelgid on Nearby Aquatic Macroinvertebrate Assemblages

Melissa A. Churchel, James L. Hanula, C. Wayne Berisford, James M. Vose, and Mark J. Dalusky

ABSTRACT

Imidacloprid, a systemic insecticide that acts on the nervous system, is currently being used to control hemlock woolly adelgid (*Adelges tsugae* Annand), which is damaging hemlock trees. The objective of this study was to determine whether soil injection with imidacloprid for hemlock woolly adelgid control near streams adversely affects aquatic invertebrates. Eastern hemlocks (*Tsuga canadensis*) in the watersheds surrounding four streams in the southern Appalachian region of Georgia and North Carolina were treated with imidacloprid. Addie Branch was the only stream that exhibited a possible effect from imidacloprid treatment. However, the data followed the same pattern as the other treatment streams, but with a more pronounced decrease in taxa due to adult emergence. Only a trace amount of imidacloprid was detected in one water sample from Holcomb Tributary over a period of 2 years, and no effect was observed on the aquatic macroinvertebrates in that stream. However, caution should be used when applying these results to other areas with different soil types (e.g., low organic matter content) that may not bind imidacloprid as tightly. Our results indicate that soil injections of imidacloprid can safely be used in the southern Appalachian area to control hemlock woolly adelgid.

**Keywords:** streams, nontarget, aquatic insects, pesticides

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae) was first detected in the eastern United States in Virginia in 1951, but apparently it remained at low levels until the 1960s. Since then, the spread of the hemlock woolly adelgid (HWA) has been rapid (McClure et al. 2001), and currently all of the eastern states from Maine to Georgia have well-established and expanding infestations. HWA feeds at the base of hemlock needles on the new twig tissue, causing needles to desiccate. By feeding on the tree's starch reserves, HWA reduces the production of new shoots and needles. Tree mortality can occur in as little as 2–4 years or more than 10 years, depending on infestation levels and other environmental stressors (McClure et al. 2001, US Forest Service 2005).

Eastern hemlock (*Tsuga canadensis* [L.] Carr.) is an important component of southern Appalachian forests, where it fills a unique ecohydrological role because of year-round water use, with the highest transpiration rates in the spring (Ford and Vose 2007). Its loss could cause increased discharge and a decrease in the daily amplitude of streamflow (Ford and Vose 2007). The shade provided by hemlocks reduces variability in stream temperatures throughout the year, resulting in cooler summer temperatures and warmer winter temperatures compared with streams draining hardwood forests (Snyder et al. 2002). In addition, streams in watersheds dominated

by hemlocks are less susceptible to drought disturbances (Snyder et al. 2002). The presence of hemlocks also affects species composition of fish and macroinvertebrates in surrounding streams. Streams draining hemlock forests support significantly more taxa than streams draining hardwood forests, including taxa found only in hemlock drainages (Snyder et al. 2002).

Current HWA control includes biological control, using known predators, and chemical control. The most effective chemical control appears to be imidacloprid, a systemic insecticide that acts on the nervous system of insects. Imidacloprid may be applied as a trunk injection directly into the tree, or to the soil via soil drenches or injections. All three application methods have been shown to significantly reduce adelgid populations (Webb et al. 2003, Doccola et al. 2005, McAvoy et al. 2005). Trunk injections appear to work more quickly than soil injections, but soil injections may be more effective and provide a longer period of HWA control (Tattar et al. 1998, Silcox 2002, Cowles et al. 2006). Movement of imidacloprid in the soil varies depending on environmental conditions. The adsorption of imidacloprid to soil particles increases with increasing organic matter content (Cox et al. 1997, Capri et al. 2001, Liu et al. 2002, 2006) and residence time (Oi 1999). The half-life of imidacloprid in soil has been reported to range from 19 to 1230 days

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This article uses metric units; the applicable conversion factors are: millimeter (mm): 1 mm = 0.039 in.; centimeters (cm): 1 cm = 0.39 in.; meters (m): 1 m = 3.3 ft; square meters (m<sup>2</sup>): 1 m<sup>2</sup> = 10.8 ft<sup>2</sup>; liter (L): 1 L = 1.057 quart; milliliter (mL): 1 mL = 0.27 fluid dram; microliter (μL): 1 μL = 0.00027 fluid dram.

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**Table 1. Lethal concentrations of imidacloprid to select aquatic organisms ( $\mu\text{g L}^{-1}$ ).**

Organism	24-h LC <sub>50</sub>	48-h LC <sub>50</sub>	96-h LC <sub>50</sub>
<b>Invertebrates</b>			
<i>Daphnia magna</i> (water flea)	>3,200,000 <sup>a</sup>	64,873 <sup>a</sup> 10,440 <sup>b</sup>	
<i>Chydorus sphaericus</i> (water flea)	161,950 <sup>a</sup>	132,673 <sup>a</sup>	
<i>Cyprretta seuratti</i> (seed shrimp)	732 <sup>a</sup>	301 <sup>a</sup>	
<i>Cypridopsis vidua</i> (seed shrimp)	>4,000 <sup>a</sup>	715 <sup>a</sup>	
<i>Ilyocypris dentifera</i> (seed shrimp)	1,122 <sup>a</sup>	517 <sup>a</sup>	
<i>Artemia</i> spp. (brine shrimp)		361,230 <sup>b</sup>	
<i>Americamysis bahia</i> (opossum shrimp)			38–159 <sup>c</sup>
<i>Lumbriculus variegates</i> (oligochaete)			6.2 <sup>d</sup>
<i>Epeorus longimanus</i> (mayfly)	2.1 <sup>d</sup>		0.65 <sup>d</sup>
<i>Simulium vittatum</i> (blackfly)		6.75–9.64 <sup>e</sup>	
<i>Aedes aegypti</i> (mosquito)		44–45 <sup>b</sup>	
<i>Aedes taeniorhynchus</i> (mosquito)		13 <sup>b</sup>	
<i>Chironomus tentans</i> (midge)			5.40–5.75 <sup>f,c</sup>
<i>Hyalella azteca</i> (amphipod)			17.44–65.43 <sup>f,c</sup>
<b>Amphibians</b>			
<i>Rana limnocharis</i> tadpoles	235,000 <sup>g</sup>	165,000 <sup>g</sup>	82,000 <sup>g</sup>
<i>Rana N. Hallowell</i> tadpoles	268,000 <sup>g</sup>	219,000 <sup>g</sup>	129,000 <sup>g</sup>
<b>Fish</b>			
<i>Cyprinodon variegates</i> (sheepshead minnow)			163,000 <sup>c</sup>
<i>Lepomis macrochirus</i> (bluegill)			>105,000 <sup>c</sup>
<i>Oncorhynchus mykiss</i> (rainbow trout)			>83,000 <sup>c</sup>

<sup>a</sup> Sanchez-Bayo and Goka 2006.<sup>b</sup> Song et al. 1997.<sup>c</sup> EPA Office of Pesticide Programs 2000.<sup>d</sup> Alexander et al. 2007.<sup>e</sup> Overmyer et al. 2005.<sup>f</sup> Stoughton et al. 2008.<sup>g</sup> Feng et al. 2004.

(Baskaran et al. 1999, Capri et al. 2001, Sarkar et al. 2001, Graebing and Chib 2004, Singh and Singh 2005, Horwood 2007). Imidacloprid is highly soluble in water ( $610 \text{ mg L}^{-1}$ ) (Krohn and Hellpointner 2002), where its half-life ranges from 1.2 hours to 46.31 days depending on the presence of light, pH of the water, and the imidacloprid formulation (Moza et al. 1998, Sarkar et al. 2001). However, the half-life of imidacloprid in river water has been estimated to be 3 days (Redlich et al. 2007).

Although injecting imidacloprid directly into the soil minimizes the risk to humans and other animals, there is concern about the pesticide reaching nearby streams. Some research has focused on the lethal concentrations of imidacloprid to various aquatic organisms (Table 1), but there have been few field experiments involving operational imidacloprid treatments. The overall aim of this study was to determine whether soil injection of imidacloprid for hemlock woolly adelgid control near streams adversely impacted aquatic invertebrates. Specifically, we examined whether imidacloprid was detrimental to aquatic invertebrates immediately following soil injection, what the long-term effects on aquatic invertebrates were, and if there was an impact, what the recovery time was for aquatic invertebrate assemblages. It was hypothesized that if imidacloprid entered the streams, it would significantly reduce the macroinvertebrate assemblage.

## Methods

### Study Sites

All of the experimental sites were within the Blue Ridge province of the southern Appalachian region. This area is characterized by dense forests and steep slopes. Rainfall is abundant, with an average annual precipitation of 179–237 cm recorded at the Coweeta Hydrologic Laboratory, Otto, NC. The dominant soils

of the treatment areas are sandy loam and loam. The percentages of sand, silt, clay, and organic matter are 23–83, 9–42, 9–48, and 0–15%, respectively (USDA Natural Resources Conservation Service). The duff layer has an average depth of 7.96 cm. Portions of the drainage basins of three headwater streams in the Chattahoochee National Forest of northeastern Georgia and one headwater stream at the Coweeta Hydrologic Laboratory in North Carolina were selected for treatment sites. The Georgia sites were located in the Chattooga River Basin and included an unnamed tributary of Holcomb Creek, Addie Branch, and Billingsley Creek (Figure 1). All three streams are tributaries of Holcomb Creek. The North Carolina site was located in the Dryman Fork basin. An adjacent stream in the Dryman Fork basin was selected to serve as the reference condition (Figure 1). Physical characteristics of the study sites are described in Table 2.

### Insecticide Treatment

On Nov. 1, 2005, 60 trees at each site in the Chattahoochee National Forest were treated with imidacloprid (Merit 75 WSP). A 910-m section of each stream with approximately 30 trees on each side of the stream was treated with soil injections using Kioritz Soil Injectors. One gram of imidacloprid, in a 10-mL solution, was injected per 2.5 cm of diameter of each tree, using the basal injection method. Injections were 4 cm deep into the duff layer and were evenly spaced in a circle 15–30 cm from the base of each tree. On May 17, 18, and 19, 2006, a 610-m section of Dryman Fork at Coweeta Hydrologic Laboratory was treated. All trees with a diameter greater than 25 cm at the base, within 15 m on each side of the stream were treated with imidacloprid. One hundred-nine trees were treated with soil injections as described above. An additional 88 trees that were next to the stream or in contact with surface water were trunk injected with imidacloprid using Mauge II Generation

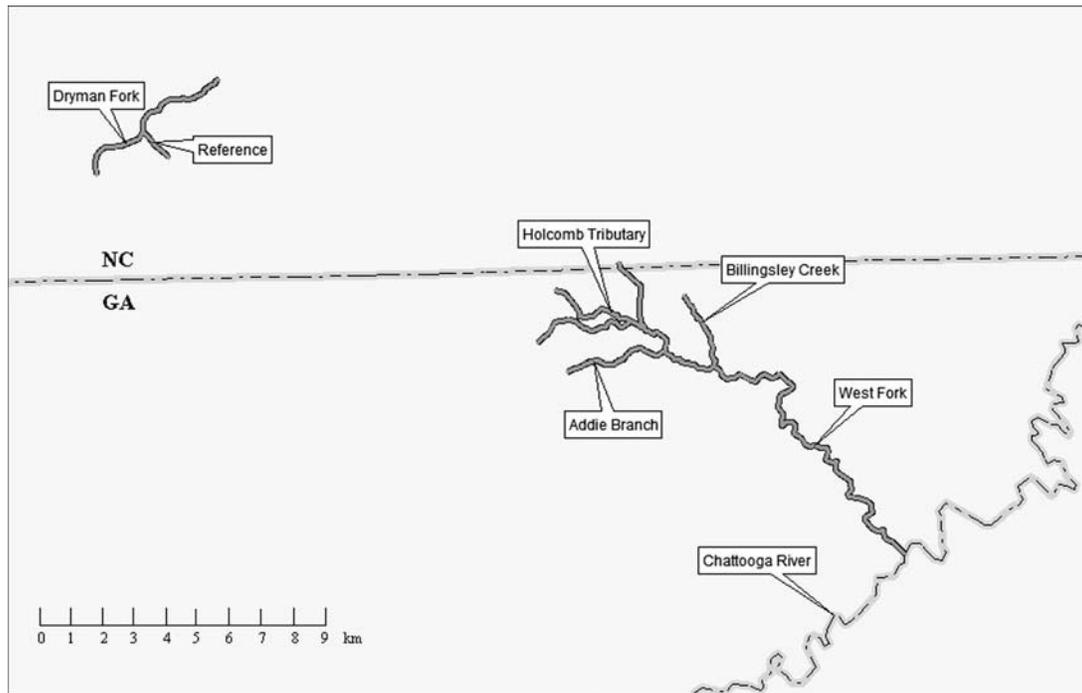


Figure 1. Study sites in Chattahoochee National Forest and Coweeta Hydrologic Laboratory.

Table 2. Physical description of study sites.

Stream name	Stream length (km)	Watershed area (km <sup>2</sup> )	Watershed perimeter (km)	Minimum elevation	Maximum elevation	Average stream width
Holcomb Tributary	2.31	1.97	6.34	696	1,070	1.69
Addie Branch	3.87	3.10	8.83	686	1,409	1.05
Billingsley Creek	2.59	2.61	7.49	650	969	2.96
Dryman Fork	2.19	2.02	6.38	865	1,476	3.79
Reference	1.36	1.90	2.56	865	1,201	4.25

Tree Injectors. The watershed adjacent to the Dryman Fork site was used as the reference condition.

### Sampling Methods

Pretreatment macroinvertebrate samples were taken at the Chattahoochee National Forest on Oct. 10, 2005 and at Coweeta Hydrologic Laboratory on Apr. 10, 2006. Posttreatment sampling at Coweeta began 2 weeks after treatment, and samples were taken biweekly for the first 3 months and then monthly through November 2007 to determine the immediate impact of imidacloprid treatment. Sampling at Chattahoochee National Forest sites began 5 months after treatment, and samples were taken monthly through November 2007 to determine the long-term impact of imidacloprid treatment. Because of a severe drought and the complete drying of Addie Branch, the last sample taken there was in August 2007. A 100-m reach was used for aquatic macroinvertebrate sampling, with the uppermost boundary approximately 10 m downstream from the nearest treated tree (Barbour et al. 1999). Four randomly selected riffles in the study reach were sampled in each of the five streams. Riffles were sampled using a Surber sampler with a fixed sampling area of 0.09 m<sup>2</sup> (Adamus 1984). The Surber sampler was placed into the streambed, and all of the contents down to 5 cm were washed into the mesh collection bag. Large cobble was scrubbed in the

field to remove any invertebrates and then placed back into the stream. Samples were preserved in 95% ethanol and returned to the lab where they were elutriated to separate the organic and inorganic material. After separation, all macroinvertebrates were identified to genus or the lowest taxonomic category possible.

Water samples were taken on each macroinvertebrate sampling date beginning 2 weeks after treatment for chemical analyses. A grab sample was taken at each site downstream of the treatment area using a 1,000-mL glass bottle. Samples were also taken in the reference stream at the same sampling time to ensure that no contamination occurred. Water samples were stored in a cold room at 5°C until chemical analyses were completed. Analysis was performed by the University of Georgia Pesticide and Hazardous Waste Laboratory using the following procedure (P. Bush, laboratory director, pers. comm.). A 500-mL sample was poured into a 1-L separatory funnel, and 75 mL of methylene chloride was added. The solution was shaken and allowed to separate into layers. The methylene chloride layer was drained off and the above process was repeated two more times with all of the drained methylene chloride layers combined. Sodium sulfate was added to the flask and swirled to remove excess water. The extract was filtered to remove sodium sulfate and concentrated under a stream of nitrogen (Zymark TurboVap) to 0.5 mL of acetonitrile. Imidacloprid residue levels were quantified by high-performance liquid chromatography (Hewlett Packard 1100

**Table 3.** Average number of macroinvertebrate taxa in treatment and reference streams prior to imidacloprid treatment and following treatment. Holcomb Tributary, Addie Branch, and Billingsley Creek were treated in November 2005, and Dryman Fork was treated in May 2006. An asterisk (\*) indicates a significant difference from the reference stream by Kruskal-Wallis ANOVA ( $P < 0.05$ ). Analyses between seasons were only done if a treatment stream was significantly lower than the reference stream in a given season.

	2006				2007			
	Pretreatment	Spring	Summer	Fall	Winter	Spring	Summer	Fall
Holcomb Tributary	23.75	33.06*	25.88	30.75*	29.50	32.92*	34.58*	36.25
Addie Branch	21.25 <sup>a</sup>	28.75 <sup>a</sup>	21.88 <sup>a</sup>	23.17 <sup>a</sup>	24.08 <sup>a*</sup>	26.42 <sup>a</sup>	24.00 <sup>a</sup>	
Billingsley Creek	27.50	32.25*	29.38*	28.17	31.58	30.08	31.33	32.08
Dryman Fork	27.00	29.38	24.08	25.50	27.58	29.19	29.63	29.63
Reference	28.75	26.00	23.63	25.00	29.17	28.06	26.75	32.00

<sup>a</sup> Averages within the same row that share the same letter are not significantly different by Kruskal-Wallis ANOVA ( $P < 0.05$ ).

HPLC) using a 25 cm × 4.6 mm ID Supelcosil LC-18 5- $\mu$ m Supelco column. The HPLC was operated at a temperature of 24°C with a flow rate of 1 mL/minute. Injection volume was 20  $\mu$ L, and the solvent gradient was 10:90 acetonitrile:water to 90:10 acetonitrile:water. Two matrix spikes and one matrix blank were analyzed with each batch of field samples. The mean percentage recovery of imidacloprid from lab spikes from water for the entire study period was  $92.78 \pm 16.82\%$ . The method detection limit for imidacloprid in water was 0.600 pbb. There was no statistically significant degradation of imidacloprid during 10, 31, 80, or 278 days in a cold storage stability study at approximately  $5 \pm 4^\circ\text{C}$ . All field samples were extracted prior to 278 days in cold storage. The analysis was performed following the method of Miles, Inc. (1991).

### Data Analysis

Data for each treatment stream were pooled by season for comparison with the reference stream to determine the effect of the imidacloprid injections on aquatic macroinvertebrate assemblages. We calculated the following indices for each stream and each season: number of taxa, number of Ephemeroptera + Plecoptera + Trichoptera (EPT) taxa, and the North Carolina Biotic Index (NCBI). The NCBI was developed to assess the general health of aquatic macroinvertebrate communities and is specific to the southeastern United States (Lenat 1993). The NCBI is calculated as  $\text{NCBI} = \sum \text{TV}_i N_i / \text{Total } N$ , where  $\text{TV}_i$  is the tolerance value of the  $i$ th taxa,  $N_i$  is the abundance of the  $i$ th taxa, and total  $N$  is the number of individuals in the sample. Tolerance values for each taxon range from 0 to 10, and abundance values are transformed into rare (1–2 per sample), common (3–9 per sample), or abundant ( $\geq 10$  per sample), coded as 1, 3, or 10, respectively. If the indices in the treatment stream were significantly lower than in the reference stream, then within-stream seasonal variation was analyzed. Values found to be significantly lower than the reference stream were also compared with the pretreatment values.

All data failed normality tests and were subsequently  $\log(x + 1)$  transformed. Data that met normality requirements were analyzed using a one-way analysis of variance (ANOVA) with a Tukey honestly significant difference test. Data that failed to meet normality requirements after log transformations were analyzed using a Kruskal-Wallis ANOVA and multiple comparisons of mean ranks for all groups using Statistica computer software (version 8, StatSoft, Inc., Tulsa, OK).

## Results

### Water Samples

The analytical minimum detection limit was determined to be 0.6 p.b. ( $0.6 \mu\text{g L}^{-1}$ ) of imidacloprid. No imidacloprid was de-

tected in any of the samples from Addie Branch, Billingsley Creek, Dryman Fork, or the reference stream. Holcomb Tributary contained less than 1.0 p.b. of imidacloprid in the Oct. 22, 2007 sample, which was taken 720 days after treatment.

### Benthic Metrics

There were multiple significant differences in the average number of taxa between the treatment streams and the reference stream; however, most of these differences were the result of higher numbers in the treatment streams, so they did not warrant further analysis (Table 3). Only Addie Branch contained a significantly lower number of taxa than the reference stream in winter 2006/2007 ( $P = 0.046$ ), with an average of five fewer taxa. This stream contained fewer taxa than the reference stream in all seasons except for spring 2006. Analysis of the seasonal variation within Addie Branch showed that the winter 2006/2007 results were not significantly different from the previous season or the pretreatment sample, which indicates that the lower number of taxa was not the result of imidacloprid leaching. Addie Branch exhibited the same seasonal pattern as the other streams, but with a smaller increase in the number of taxa from fall to winter.

Analysis of the average number of EPT taxa provided similar results. Again, there were multiple seasons with significantly higher numbers of EPT taxa in the treatment streams than in the reference stream (Table 4). Addie Branch contained a significantly lower number of EPT taxa than the reference stream in summer 2006 ( $P = 0.008$ ). Analysis of seasonal variation showed that summer 2006 was also significantly lower than spring 2006 in Addie Branch ( $P = 0.010$ ) but not significantly different from the pretreatment sample. All streams experienced a decrease in the number of EPT taxa between spring and summer, but this decrease was more pronounced in Addie Branch, with a loss of six taxa.

NCBI scores of the treatment streams were not significantly different from those of the reference stream (Table 5). All scores were less than 4.18, which ranks them in the excellent water quality class for the mountain ecoregion.

## Discussion

The primary goals of this study were to determine the short-term and long-term effects of imidacloprid treatment on macroinvertebrate assemblages. Although there were multiple significant differences between the treatment streams and the reference stream, most of these differences resulted from the treatment streams having higher numbers than the reference stream. This may indicate that the reference stream was not ideal for this study, although this was not evident in the pretreatment samples.

**Table 4. Average number of Ephemeroptera + Plecoptera + Trichoptera taxa in treatment and reference streams prior to imidacloprid treatment and following treatment. Holcomb Tributary, Addie Branch, and Billingsley Creek were treated in November 2005, and Dryman Fork was treated in May 2006. An asterisk (\*) indicates a significant difference from the reference stream by Kruskal-Wallis ANOVA ( $P < 0.05$ ). Analyses between seasons were only done if a treatment stream was significantly lower than the reference stream in a given season.**

	Pretreatment	2006			2007			
		Spring	Summer	Fall	Winter	Spring	Summer	Fall
Holcomb Tributary	12.75	19.69*	15.50	18.83*	19.58	20.33*	20.67*	21.08
Addie Branch	10.75 <sup>a,c</sup>	16.63 <sup>b</sup>	11.13 <sup>*,a</sup>	14.33 <sup>a,b</sup>	15.25 <sup>a,b</sup>	16.33 <sup>b,c</sup>	14.08 <sup>a,b</sup>	
Billingsley Creek	15.75	18.81*	17.25*	17.08	18.50	17.92	18.08	18.50
Dryman Fork	14.75	16.75	14.92	15.94	15.83	17.00	17.38	18.38
Reference	16.25	15.63	14.42	16.13	17.58	16.81	15.63	19.50

<sup>a,b,c</sup> Averages within the same row that share the same letter are not significantly different by Kruskal-Wallis ANOVA ( $P < 0.05$ ).

**Table 5. Average North Carolina Biotic Index scores in treatment and reference streams by season. Holcomb Tributary, Addie Branch, and Billingsley Creek were treated in November 2005, and Dryman Fork was treated in May 2006.**

	Pretreatment	2006			2007			
		Spring	Summer	Fall	Winter	Spring	Summer	Fall
Holcomb Tributary	3.93	3.82	3.36	3.76	3.75	3.87	3.31	3.83
Addie Branch	3.80	3.41	3.47	3.20	3.22	3.29	3.09	
Billingsley Creek	3.51	3.94	3.55	3.60	3.91	3.94	3.59	3.83
Dryman Fork	3.90	3.63	3.27	3.81	3.72	3.80	3.51	3.87
Reference	3.58	3.07	3.12	3.55	3.50	3.29	3.15	3.66

Comparisons between the indices that were significantly different from the reference stream and the pretreatment data should help to account for the natural differences from the reference stream. Addie Branch had a significantly lower total number of taxa in winter 2006/2007 than the reference stream. Analysis of the within-stream variation showed that none of these values were significantly lower than the previous season in the same stream or the pretreatment data. Because these values did not indicate a significant loss in the invertebrate community over time, we do not believe that they represent an impact from imidacloprid treatment. Although these values differed greatly from the reference stream, they follow the same seasonal pattern observed for all of the streams in this study. In another study of the implications of pesticide exposure on aquatic invertebrates at Coweeta Hydrologic Laboratory, the NCBI and EPT values for the treatment stream illustrated the significant changes in macroinvertebrate assemblages due to insecticide exposure (Wallace et al. 1996).

The only metric that exhibited a significant difference from the reference stream and from the previous season within the same stream was the number of EPT taxa in Addie Branch in summer 2006. All streams experienced a loss in taxa between spring and summer, likely due to adult emergence. Although it is possible that the larger loss of taxa in Addie Branch was due to imidacloprid entering the stream, it is more likely due to higher variability caused by the drying of Addie Branch. If the decrease in the number of EPT taxa in Addie Branch was due to imidacloprid, the impact was relatively small and short-lived. There were still 11 EPT taxa present in summer 2006. This number is much higher than the result after treating streams at Coweeta with methoxychlor, where the number of EPT taxa dropped from 18.0 prior to treatment to 5.4 following initial treatment (Wallace et al. 1996). The number of EPT taxa in Addie Branch in the fall of 2006 increased by three taxa and was not significantly different from the reference stream or the summer 2006 sample.

Although differences occurred in the average number of taxa and number of EPT taxa, the NCBI scores for all streams and all seasons were less than 4.18, which ranks them in the excellent water quality class for the mountain ecoregion. Since no scores were outside of the excellent water quality rating, we conclude that the macroinvertebrate assemblages were not significantly affected by the application of imidacloprid.

Although a trace amount of imidacloprid was detected in the Oct. 22, 2007, sample from Holcomb Tributary, no effect on the aquatic invertebrate assemblage was observed for this stream. The amount present, less than  $1.0 \mu\text{g L}^{-1}$ , is well below the reported 50% lethal concentration ( $\text{LC}_{50}$ ) for numerous aquatic invertebrates (see Table 1). This result also is in agreement with a study carried out at Mount Lake, Virginia, where no change in the number of invertebrates occurred after exposure to  $0.5 \mu\text{g L}^{-1}$  of imidacloprid in the springs around the lake (McAvoy et al. 2005). In another study, mayfly density was severely reduced after a continuous 20-day exposure to  $0.8 \mu\text{g L}^{-1}$  of imidacloprid (Alexander et al. 2008), which suggests that the imidacloprid in Holcomb Tributary quickly dissipated. However, this amount of imidacloprid could have sublethal effects on certain invertebrates. Exposure to imidacloprid concentrations of  $0.0018 \mu\text{g L}^{-1}$  and less had significant effects on the larval development of the damselfly *Coperana annulata* (Chang et al. 2007). Twelve-hour exposures to concentrations as low as  $0.1 \mu\text{g L}^{-1}$  reduced the head length of *Baetis* mayflies and the thorax length of *Epeorus* mayflies (Alexander et al. 2008).

The application of our results to future HWA control efforts should be done with caution. For example, the use of different formulations of imidacloprid may cause different results from our study. In our study, we used Merit 75 WSP (water-soluble packets) for soil injections. Powder formulations, such as Gaucho 70 WS, have been shown to be more persistent in water and in soil than liquid formulations, such as Confidor 200 SL (Sarkar et al. 1999, 2001). When topically applied to turf, the granular formulation (Merit 0.5 G) resulted in a higher concentration of imidacloprid in

runoff than the wettable powder formulation (Merit 75 WP) (Armbrust and Peeler 2002). In sandy loam soil, leaching was greater for the water-dispersible powder (Gaucho 70 WS) than for the soluble concentrate (Confidor 200 SL), and least for the suspension or flowable concentrate (Admire 350 SC) (Gupta et al. 2002).

Differences in the composition and characteristics of the soil may also affect the potential for imidacloprid to contaminate streams. Increased soil pH can increase the half-life of imidacloprid (Sarkar et al. 1999, 2001), whereas the half-life is 44.6% shorter in moist sandy soil than dry sandy soil (Graebing and Chib 2004), but moisture has no effect on degradation soil consisting of approximately 59, 28, and 10% sand, silt and clay, respectively (Baskaran et al. 1999). Soils with high organic matter content have stronger sorption of imidacloprid than soils low in organic matter (Cox et al. 1997, Capri et al. 2001, Liu et al. 2002, Liu et al. 2006).

The soil in our treatment area consists of sandy and stony loam. According to the US Forest Service Ecological Risk Assessment, there is very low potential for imidacloprid to leach from loam or sandy loam soils. It is estimated that less than 0.001  $\mu\text{g L}^{-1}$  would enter nearby streams following rainfall (Anatra-Cordone and Durkin 2005). Imidacloprid has a sorption coefficient,  $K_d$ , between 3.40 and 16.9 in sandy loam soil (Cox et al. 1998a, 1998b, Oi 1999, Oliveira et al. 2000). These values are significantly higher than the  $K_d$  for fine sand (0.52 by Cox et al. 1998a, 1998b) and for sand (1.18 by Oliveira et al. 2000). Soil injections of imidacloprid in other soil types may pose a higher risk of contamination to nearby streams. In addition, we used shallow injections, which rarely penetrate the leaf litter/duff layer to take advantage of the imidacloprid binding properties of soil organic matter. Our study indicates that soil injections of Merit 75 WSP in the loam soils of the southern Appalachians for control of hemlock woolly adelgid does not have a significant short-term or long-term negative impact on nearby streams and the aquatic invertebrates that inhabit them.

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