Long-Term Effects of Imidacloprid on Eastern Hemlock Canopy Arthropod Biodiversity in New England

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Abstract - The systemic insecticide imidacloprid is commonly used to protect trees against attack by the *Adelges tsugae* (Hemlock Woolly Adelgid [HWA]), an invasive pest that threatens *Tsuga canadensis* (Eastern Hemlock) and *T. caroliniana* (Carolina Hemlock) in eastern North America. Although there have been some studies documenting the short-term (1–3 years) impact of imidacloprid on non-target arthropods in hemlock systems, almost nothing is known about the impact over longer time scales. Here, using a set of trees which were experimentally treated 3 and 9 years prior to this study, we found that while the impact of imidacloprid on HWA may be approaching the limits of detection and efficacy on trees treated 9 years ago, there is still an intermittently detectable impact on HWA density. Similarly, 9 years after application there is a subtle but detectable increase in arthropod richness and a shift in canopy-arthropod community composition. Results from the 3-year treated trees were, however, ambiguous, but may be the result of detectable cross-contamination of insecticide among trees.

Introduction

Adelges tsugae Annand (Hemiptera: Adelgidae; Hemlock Woolly Adelgid [HWA]), is an introduced insect pest that poses a serious threat to *Tsuga canadensis* L. Carriere (Eastern Hemlock) and *Tsuga caroliniana* (Carolina Hemlock) in eastern North America. These tree species provide a unique ecological niche for a wide diversity of flora and fauna (Ingwell et al. 2012, Jordan and Sharp 1967, Tingley et al. 2002), and are associated with changes in fish-community structure in riparian systems (Snyder et al. 2002). Stands infested with HWA commonly experience high rates of hemlock mortality, and stand structure can change rapidly (Orwig and Foster 1998, Orwig et al. 2002). In Connecticut, HWA has been present since at least 1995 and has led to hemlock-mortality rates as high as 95% (Orwig et al. 2002; Preisser et al. 2008, 2011), and the loss of this foundation species is likely to impact a diverse community of organisms associated with hemlock ecosystems (Ingwell et al. 2012).

Efforts to manage the impacts of HWA have included biological control (Onken and Reardan 2011), identification of naturally occurring resistance (Ingwell et al. 2009), the development of hybrids (Montgomery et al. 2009), silvicultural methods (Fajvan 2008), and the use of systemic insecticides (Coots 2012, Cowles and Lagalante 2009, Cowles et al. 2006). Currently, the only readily available and proven

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method for protecting individual trees is the application of insecticides, including imidacloprid. Commercial formulations labeled for use on hemlocks for the control of HWA facilitates the broad use of this insecticide, and its ease of application, low mammalian toxicity, and strong binding affinity to organic matter (Silcox 2002) have made it a valuable short-term solution for HWA infestations.

Imidacloprid can, however, persist in the environment and potentially affect a broad range of arthropods (Kreutzweiser et al. 2008, Suchail et al. 2001), raising concerns about the understudied potential for long-term impacts on non-target organisms. Previous studies have documented impacts on non-target arthropods a year after application (Dilling et al. 2009), and have shown imidacloprid can remain effective against HWA up to 4 years post-application (Eisenback et al. 2014). Past research has also found that this insecticide can be detected in plant tissues up to 8 years post-application (Cowles and Lagalante 2009). Yet, little is known about the long-term impacts of imidacloprid applications on non-target hemlock-canopy arthropods.

In this study, we sought to take advantage of trees treated with imidacloprid in an early HWA-control study to evaluate the long-term impacts of its application. Using trees treated 3 and 9 years prior to this study, we addressed 3 key questions. First, is imidacloprid (and the metabolite olefin) still detectable in treated trees 3 and 9 years post-application? Second, is there a detectable effect of imidacloprid application on HWA 3 and 9 years post application? Third, is there evidence of effects on the alpha (within tree) and beta (among trees) diversity and community structure of the canopy arthropods found in Eastern Hemlock 3 and 9 years after insecticide application? Providing this information may facilitate long-term planning and strategies based on the use of this widely used insecticide.

Methods

Study history/tree selection

The hemlock trees used in this study were originally selected as part of several previous studies designed to evaluate the efficacy of imidacloprid as a systemic insecticide to control HWA in Eastern Hemlock (Cowles et al. 2006). The first of these was established in 2002, when 28 HWA-infested Eastern Hemlock trees in Shenipsit State Forest (Stafford, CT, 41.96322N, 72.40436W) were used to evaluate the efficacy of multiple methods and seasons of imidacloprid application. Twenty-four trees were treated with imidacloprid using one of several application methods, the remaining 4 trees were used as controls (full description available in Cowles et al. 2006). In this original study, trees were treated in the fall of 2002 or the spring of 2003; however, seasonality was not found to play a significant role in insecticidal efficacy (Cowles et al. 2006), and so we consider the trees as a single cohort. In the spring and summer of 2011, we sampled 18 of the original study trees and 22 interspersed untreated trees (two of which were original control trees) of similar size and canopy position; we sampled them. For our samples, we collected canopy arthropods and foliage for insecticide-residue analysis, and undertook HWA-population surveys as described below. The time interval between insecticide treatments made to these trees and our sampling represents a unique opportunity to evaluate the long-term impacts of imidacloprid on non-target canopy arthropods.

A second group of trees was treated 3 years prior to the current study as part of an evaluation of multiple insecticides (including imidacloprid) for use in controlling HWA. The experimental trees were located in a windbreak at the Connecticut Agricultural Experiment Station Griswold Research Center (Voluntown, CT; 41.560197N, 71.876225W) (R. Cowles, unpubl. data). We selected trees from this group that were treated in 2008 with a single application of imidacloprid. The treatment consisted of 1 g of active ingredient per 2.5 cm DBH, injected into the soil within 30 cm of the base of the trunk using a Kioritz soil injector (Yamabiko Corp, Ome, Japan). Control trees were selected from the untreated trees within the same windbreak. To reduce the potential for contamination by applications made to nearby trees (trees within the windbreak were within 2–3 m of neighboring trees), we did not select untreated trees adjacent to trees which received chemical treatment, producing a minimum treated-control inter-tree distance of 6 m. We further limited study trees to those trees with adequate foliage for complete sampling. The selection criteria used yielded 15 treated trees and 14 control trees. The 2 sets of study trees treated in 2002 and 2008 are hereafter referred to as the 3-year and 9-year trees.

Imidacloprid analyses

In October 2011, we quantified imidacloprid and olefin levels in the needles from each of the treated and untreated trees in the 3- and 9-year groups. We collected 4 branches, one from each of 4 orthogonal directions, from each tree. The four branches were then combined and dried in paper bags for 2 months at room temperature (~24 °C). We then dislodged the dried needles from the branches, and ground 5–10 g of needles using a Wiley mill and a #40 screen. We added 5 ml of methanol to 0.5 g of the ground foliage and agitated the mixture on a platform rocker for 24 hours. Samples were then passed through a 0.2-µm filter, and a 0.5-ml aliquot was placed in an amber vial and sent to Dr. Anthony Lagalante of Villanova University, Villanova, PA, where it was analyzed for imidacloprid and olefin concentrations using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Additional details regarding these methods are available in Eisenback et al. (2010).

HWA survey

In January 2012, we assessed HWA density (sistens generation) on both the 9-year and 3-year trees. We sampled HWAs at this time because HWA matures and feeds during the winter, so individuals sampled in mid-winter had been exposed to the foliage and insecticide concentrations found in the foliage sampled the previous fall. Because HWA populations on a tree are often heterogeneously distributed (Evans and Gregoire 2007), we collected 5 randomly selected branches within reach of the ground from each tree. For each branch, we counted both dead and live HWA on 5 of the outermost stems (first bud-scale scar to current bud) with live buds and recorded the length of the foliage surveyed. Using this portion of the foliage limited sampling to live stems on only the most recent growth. We pooled values for each tree to produce a tree-level HWA density (HWA/cm) value.

Because HWA densities were low in the winter of 2011/2012, we repeated HWA surveys in February 2013. However on these branches, we counted only live HWA,

which produce conspicuous wax-like secretions and are the survivors of the overwintering sistens generation.

Arthropod-community sampling

We conducted canopy-arthropod surveys in mid-summer (9–17 August 2011). Arthropods were collected from 2 branches of each tree using 1 of the following 2 methods. We sampled the first branch using a standard beat-sheet method in which a 1-m^2 sheet (Bioquip Products Ripstop Beating Sheet, Rancho Dominguez, CA) was placed under a branch with approximately 1 m^2 of foliage. We rapidly struck the branch 20 times with a 0.5-m piece of 1-inch PVC pipe to dislodge arthropods. There was a 3-cm hole at the center of the beat sheet with a 3 cm x 7 cm collection vial suspended below into which arthropods could slide. We used an aspirator to collect arthropods that did not quickly slide into this central vial. We sampled the second branch using a method that mirrored the first, except that prior to beating the branch, we fogged the branch with a knockdown fogging agent containing 1% pyrethrin (Pyrocide®100). The fog was applied using a commercially available home-owner-style propane-powered fogger (Fountainhead Group Inc., New York Mills, NY, models Burgess and Black Flag) modified with the addition of 61 cm of 10 cm-diameter flexible corrugated aluminum tube (dryer venting) with a 90° vertical bend that directed the fog upwards towards the branch. We added the fogging agent to augment the standard beat-sheet method which might otherwise have failed to capture more active, winged arthropods. We used pyrethrin because it has low mammalian toxicity and relatively short persistence (Crosby 1995, Kaneko 2011). Sampled branches were within 3 m of ground level. Because estimates of species richness are highly dependent on sampling effort (Schoener 1976, Trotter and Whitham 2011), we omitted from the analyses data from trees with canopies from which we were only able to sample a single branch, and so obtained a sample size of 15 treated and 14 control trees in the 3-year group. Each of the 18 treated and 22 control trees in the 9-year group yielded complete samples.

Statistical analyses

Because the 3-year and 9-year trees were separated by ~63 km, we expected a high level of variation in community composition which would be unrelated to imidacloprid, but rather to geographic differences. Thus, we limited our analyses of HWA densities and arthropod community composition to comparisons of trees within locations (i.e., within the 3- and 9-year groups).

We compared imidacloprid concentrations in needles between treated trees in the 3-year and 9-year groups of trees using a Student's *t*-test (R Core Team 2014). Comparisons of HWA densities between treated and control trees within the 3- and 9-year groups were made using the non-parametric Wilcoxon ranked-sum test (R Core Team 2014).

Three measures of arthropod community structure—abundance, morphospecies richness, and community composition—were compared between treated and control trees within the 3- and 9-year tree groups. We compared individual abundance and morphospecies richness between the treated and control trees using the Wilcoxon

ranked-sum test, and our results are presented using medians and an interquartile range (distance between 25th and 75th percentile) as measures of central tendency and dispersion. Abundance and morphospecies richness are dependent on sampling effort, so we generated rarefaction curves to qualitatively compare morphospecies richness (beta diversity or species turnover) in the 3-year and 9-year trees using EstimateS version 9 (Colwell 2013) with 1000 randomizations.

Differences in community composition between treated and control trees were graphically depicted using non-metric multidimensional scaling (NMDS) based on Sorensen Bray-Curtis distances (McCune and Grace 2002) using random starting coordinates in 250 runs. We employed the non-parametric multi-response permutation procedure (MRPP) to statistically compare dissimilarities in community composition (using Sorensen Bray-Curtis distances) between treated and control trees within the 3-year and 9-year groups (McCune and Grace 2002). Indicator-species analysis was conducted to identify morphospecies that had a high abundance and fidelity associated with treated or control trees using 4999 permutations. NMDS, MRPP, and indicator-species analyses were conducted using PCOrd (McCune and Mefford 2006).

Post hoc analyses

Eight treated trees in the 9-year group did not have detectable levels of imidacloprid, but 2 control trees in the 3-year group did, thus, we repeated the previously described comparisons and statistical analyses as post-hoc tests, using the presence/absence of imidacloprid as an a-posteriori grouping variable.

Results

Imidacloprid analyses

Within the 9-year group, 10 of the 18 treated trees had detectable levels of imidacloprid, while in the 3-year group all of the treated trees, and 2 of the untreated control trees had detectable levels of imidacloprid. We removed these 2 contaminated control trees from analyses of community composition and HWA abundance, resulting in a reduction in the number of control trees from 14 to 12. As we expected, imidacloprid-residue levels were higher in the more recently treated 3-year trees compared to the 9-year treated trees ($t = 6.2695$, df = 14, $P = 0.00002$; Table 1).

HWA surveys

In January 2012, trees treated with imidacloprid 3 years prior to the study had lower total HWA densities than control trees ($W = 2$, $P < 0.0001$; Fig. 1A). However, comparison of the treated and control trees in the 9-year group revealed no statistically significant differences in HWA density ($W = 146$, $P = 0.1598$), though the trends were similar to those observed in the 3-year group. When we surveyed the trees in February 2013, the overall density of live HWA was higher in the untreated trees in both the 3- and 9-year groups (3-year $W = 11$, $P < 0.0001$; 9-year $W = 124$, $P = 0.0457$; Fig. 1B).

Arthropod community structure

Within the 3-year group, treated trees had a median of 78 arthropods (interquartile range = 75, n = 15) from a median of 30 morphospecies (interquartile range = 20), while control trees had a median of 103 arthropods (interquartile range = 85.5, n = 12) from a median of 32.5 morphospecies (interquartile range = 15.75). Neither richness nor abundance differed significantly (Wilcoxon: richness W = 75, P = 0.479; abundance W = 71, P = 0.373; Fig. 2A, B), indicating no differences in median alpha diversity between control trees and trees treated with imidacloprid 3 years prior.

Table 1. Imidacloprid levels detected in treated and control trees treated 9 and 3 years prior.

	Mean imidacloprid concentration (ppb) ± SE	
	Treated tree	Control tree
9-year trees	3.76 ± 5.04 (n = 18)	0.00 ± 0.00 (n = 22)
3-year trees	122.82 ± 73.41 (n = 15)	0.00 ± 0.00 (n = 12)

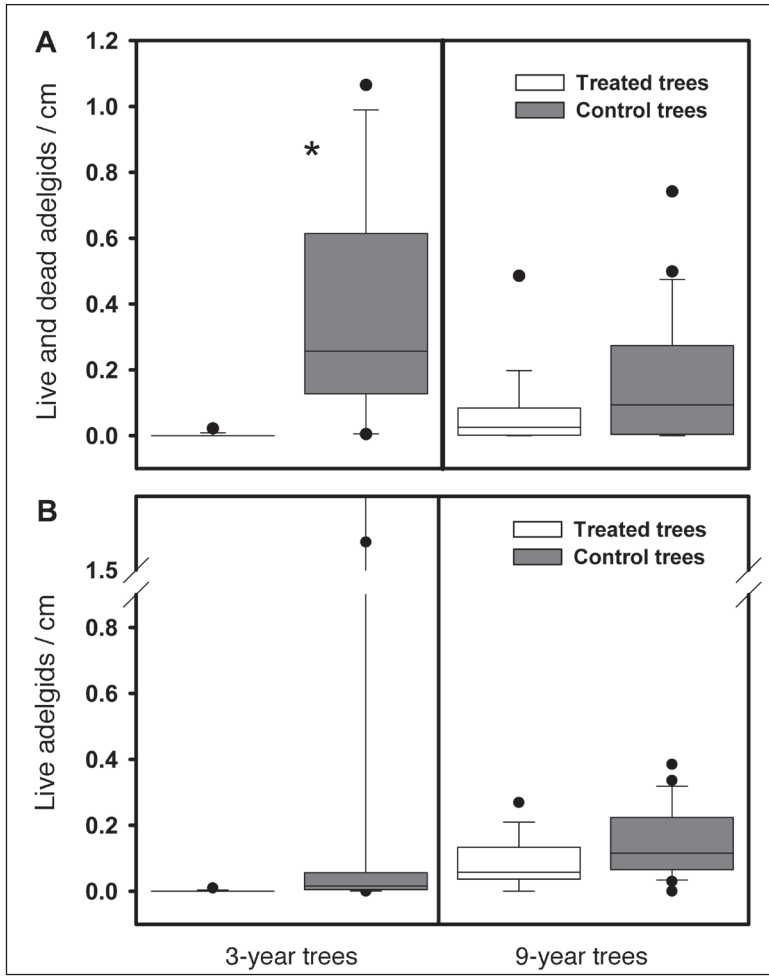

Figure 1. Box-and-whisker plots showing the median (dividing line in box), quartile (upper and lower ends of box), 10th and 90th percentiles (ends of lines), and outliers (dots) for: (A) HWA densities (both live and dead HWA combined) in the 3-year and 9-year groups in January 2012, and (B) Live HWA densities in the 3-year and 9-year groups in February 2013. The symbol * indicates statistical significance ($P < 0.05$) using a Wilcoxon ranked-sum test.

Sampled trees in the 9-year post-treatment group did however, exhibit a difference in median morphospecies richness, though the directionality was unexpected. Among the 9-year post-imidacloprid-application trees, treated trees had a median of 33 arthropod morphospecies (interquartile range = 13, n = 18); control trees yielded a median of 29 morphospecies (interquartile range = 7.25, n = 22, W = 274.5, P = 0.0385; Fig. 2C, D). The median arthropod abundance did not differ between treated (120, interquartile range = 90) and control trees (86.5, interquartile range = 43) in the 9-year group (W = 254.5, P = 0.128). Beta diversity (variation in species composition from one tree to the next) was also very similar between treated and control trees in both the 3-year and 9-year groups (Fig. 3), and the curves indicated the sample effort/intensity was similar between the groups at both locations.

Comparison of community composition among the 3-year trees by ordination (NMDS, 2 dimensions) showed substantial overlap in community structure (Fig. 4A) between treated and control trees, with no statistical difference (MRPP P = 0.374). However, indicator-species analysis in the 3-year group identified 1 morphospecies with a statistically significant association with control trees (Table 2).

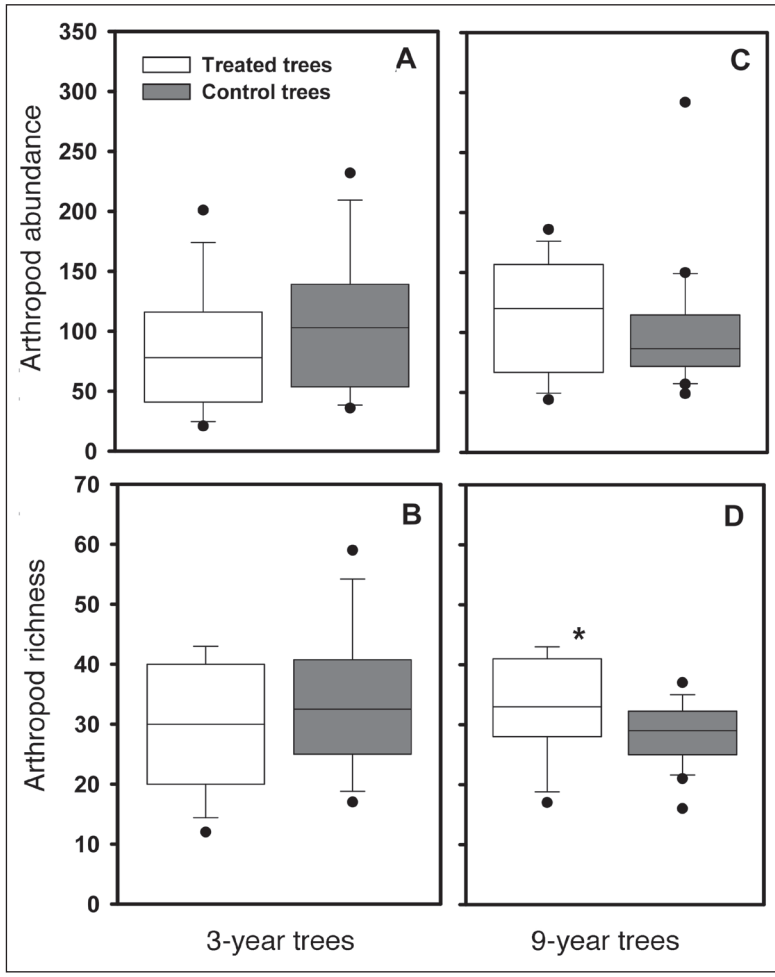

Figure 2. Box-and-whisker plots showing the median (dividing line in box), quartile (upper and lower ends of box), 10th and 90th percentiles (ends of lines), and outliers (dots) for: arthropod abundance (top) and richness (bottom) in treated and control trees in the 3-year (left) and 9-year (right) groups. The symbol * indicates statistical significance ($P < 0.05$) using a Wilcoxon ranked-sum test.

In contrast to the findings in the 3-year group, community structure among the 9-year trees differed between treated and control groups (MRPP $P = 0.041$), though ordination suggested the communities' composition included substantial overlap (NMDS, 2 dimensions; Fig. 4B). Indicator-species analysis also highlighted differences in the communities; treated trees yielded 5 indicator morphospecies, and control trees had 4 (Table 2).

Post hoc analyses

The lack of a difference in median alpha diversity between treated and control trees in the 3-year group initially appeared to be in conflict with previously published data (Dilling et al. 2009) as did the directionality of the observed differences in alpha diversity in the 9-year group. As mentioned previously, analysis of foliage

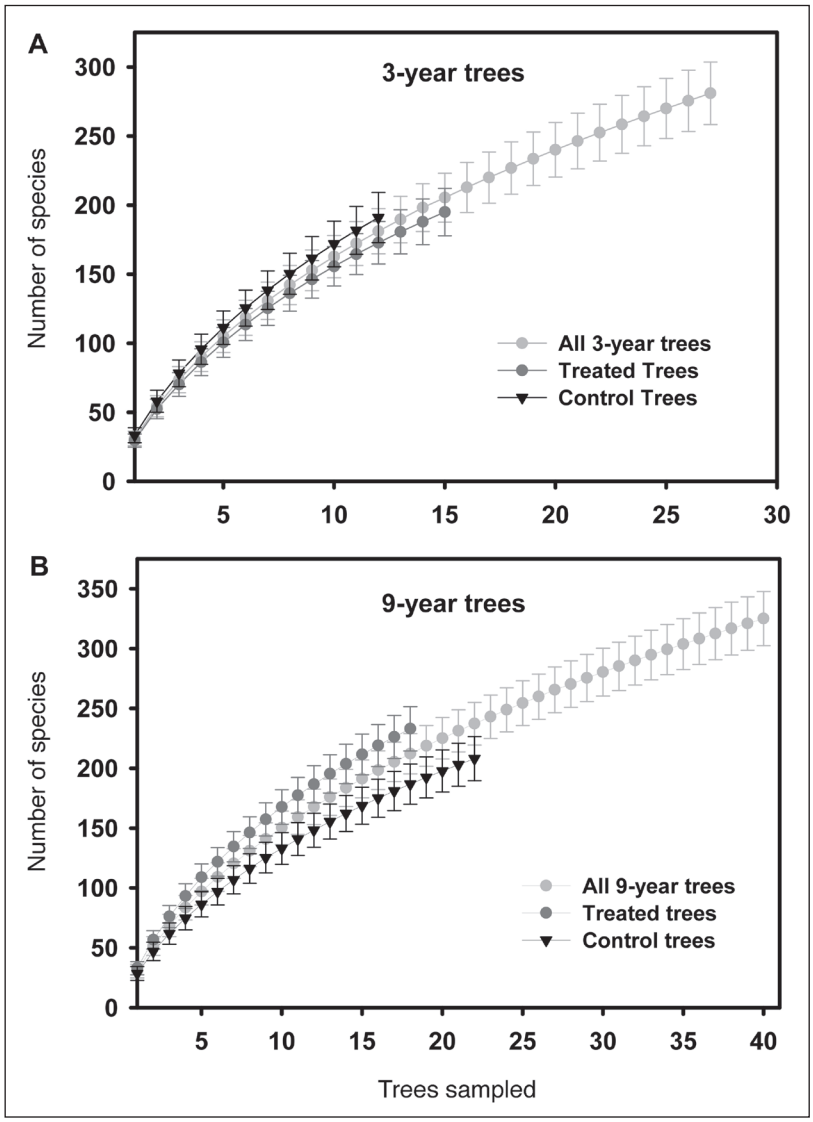

Figure 3. Species-accumulation curves rarified by sample unit (trees) for treated and control trees in the 3-year (A) and 9-year (B) groups. Vertical lines represent the 95% confidence intervals.

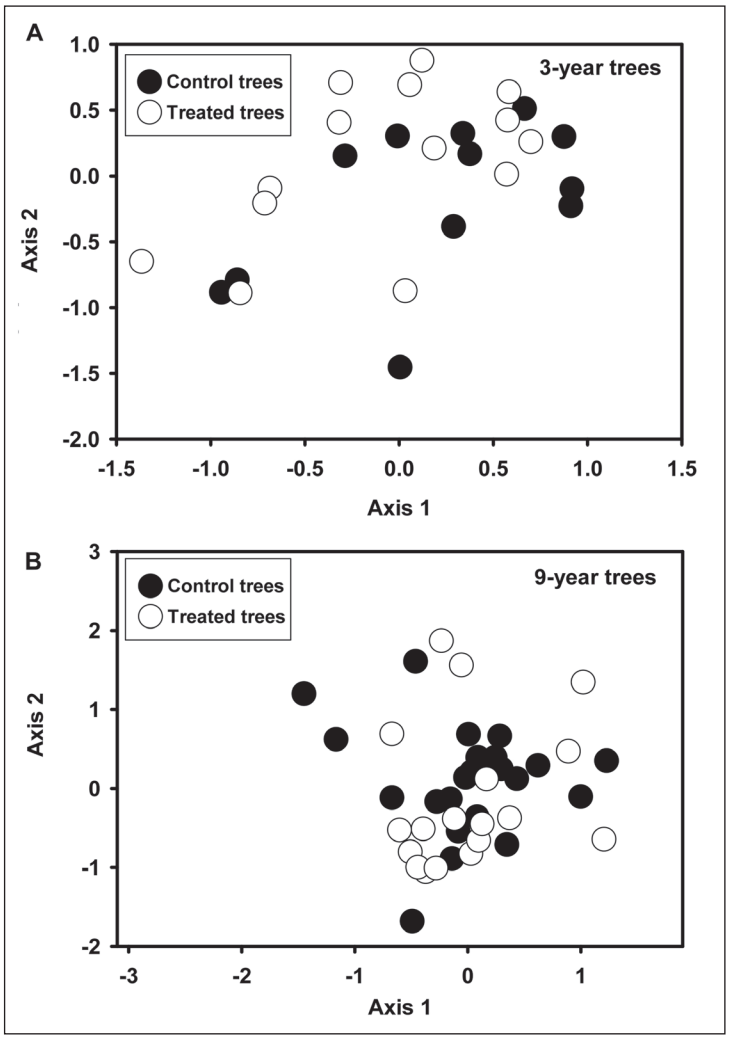

Figure 4. NMDS ordinations based on canopy arthropods in treated and control trees in the 3- (A) and 9-year (B) groups.

Table 2. Indicator species for treated and control trees treated 9 and 3 years prior. Indicator value is a composite of the value of % perfect indication based on abundance and fidelity. Mean IV is from randomized groups.

Morphopecies (ID number)	Observed indicator value (IV)	Mean IV (± SD)	*P*-value	Imidacloprid	Application
Araneae (107)	41.7	17.1 (± 6.64)	0.011	Control	3-year
Lepidoptera (03)	34.0	19.1 (± 5.43)	0.023	Treated	9-year
Araneae (034)	39.1	17.6 (± 5.48)	0.006	Treated	9-year
Araneae (115)	26.1	13.6 (± 5.08)	0.048	Treated	9-year
Araneae (147)	38.2	20.9 (± 6.06)	0.016	Treated	9-year
Araneae (162)	31.3	16.5 (± 5.43)	0.015	Treated	9-year
Psocoptera (13)	50.6	36.5 (± 6.35)	0.034	Control	9-Year
Thysanoptera (06)	59.7	43.8 (± 5.87)	0.016	Control	9-year
Lepidoptera (14)	31.5	17.4 (± 5.48)	0.023	Control	9-year
Araneae (241)	52.2	28.0 (± 6.54)	0.005	Control	9-year

from the sampled trees revealed that in the 3-year group, 2 untreated trees had detectable levels of insecticide, while imidacloprid was not detected in 8 of the 9-year treated trees. To address this issue, we re-analyzed the data a posteriori with trees assigned to groups based on the presence or absence of detectable imidacloprid.

Using these a posteriori groups, HWA densities were significantly lower in trees with detectable imidacloprid levels in both the 3-year and 9-year groups in both 2012 (Wilcoxon: 3-year $P < 0.0001$, 9-year $P < 0.0356$) and 2013 (Wilcoxon: 3-year $P < 0.0001$, 9-year $P = 0.0076$) (Fig. 5A, B).

The 3-year trees yielded a median of 30 (interquartile range = 19; $n = 17$) morphospecies and 78 (interquartile range = 64) individual arthropods on trees with imidacloprid. Trees without imidacloprid had a median of 32.5 (interquartile range = 15.75; $n = 12$) morphospecies and 103 (interquartile range = 85.5) individual arthropods (Fig. 6A, B). However, similar to the results of our a priori analyses, there

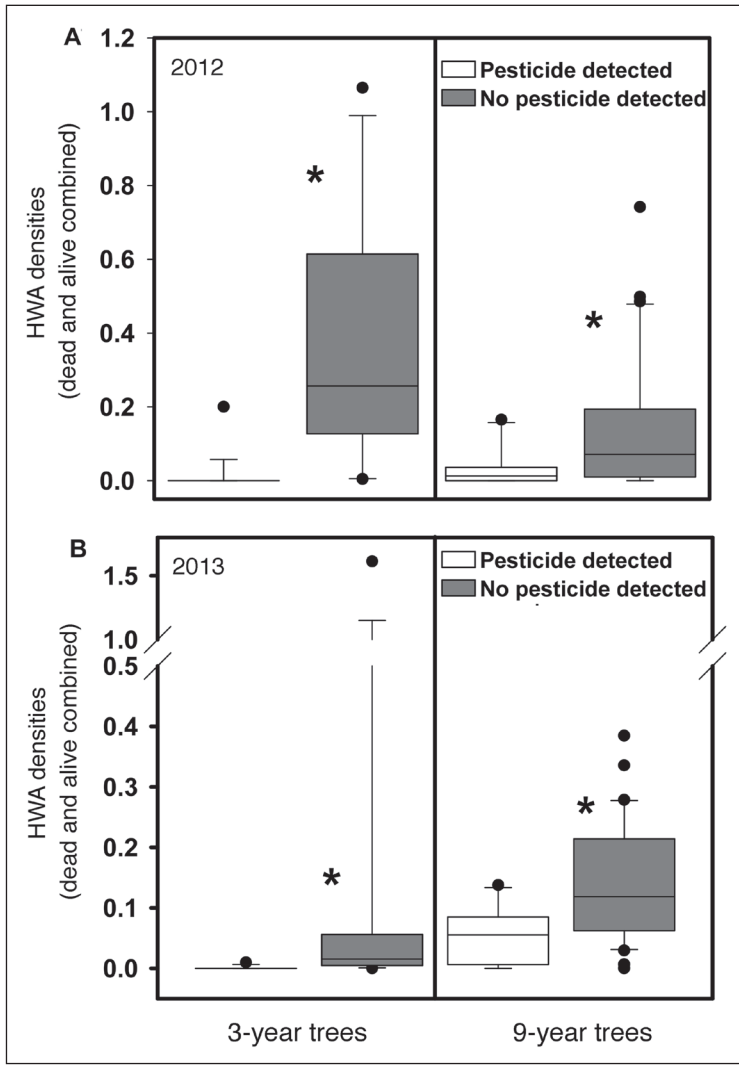

Figure 5. Box-and-whisker plots showing the median (dividing line in box), quartile (upper and lower ends of box), 10th and 90th percentiles (ends of lines), and outliers (dots) for: HWA densities (both live and dead HWA combined) in 2012 (A), and 2013 (B) on trees treated 3-years and 9-years prior. The symbol * indicates statistical significance ($P < 0.05$) using a Wilcoxon ranked-sum test.

were no statistically significant differences in canopy-arthropod morphospecies abundance ($W = 86$, $P = 0.4919$) or richness ($W = 80.5$, $P = 0.3524$).

Arthropod abundances among the 9-year trees with and without detectable levels of imidacloprid remained statistically indistinguishable when grouped a posteriori. Trees with detectable imidacloprid yielded a median of 120 (interquartile range = 59.5; $n = 10$) arthropods; trees without detectable imidacloprid yielded a median of 88 (interquartile range = 51; $n = 30$) arthropods ($W = 199$, $P = 0.1297$; Fig. 6C). The difference in morphospecies richness between treated and control 9-year trees detected using the a priori groups remained when based on the a posteriori grouping. Trees with detectable levels of imidacloprid had a median of 36.5 (interquartile range = 9.5) morphospecies, and trees without detectable imidacloprid had a median of 29.5 (interquartile range = 8.25) morphospecies ($W = 231$, $P = 0.0118$; Fig. 6D), indicating an increase in median alpha-level diversity associated with detectable imidacloprid.

Surprisingly, for both the 3-year and 9-year trees, a post-hoc analysis of community composition did not detect a significant difference between the a posteriori tree groups (MRPP: $P = 0.241$ and $P = 0.160$, respectively).

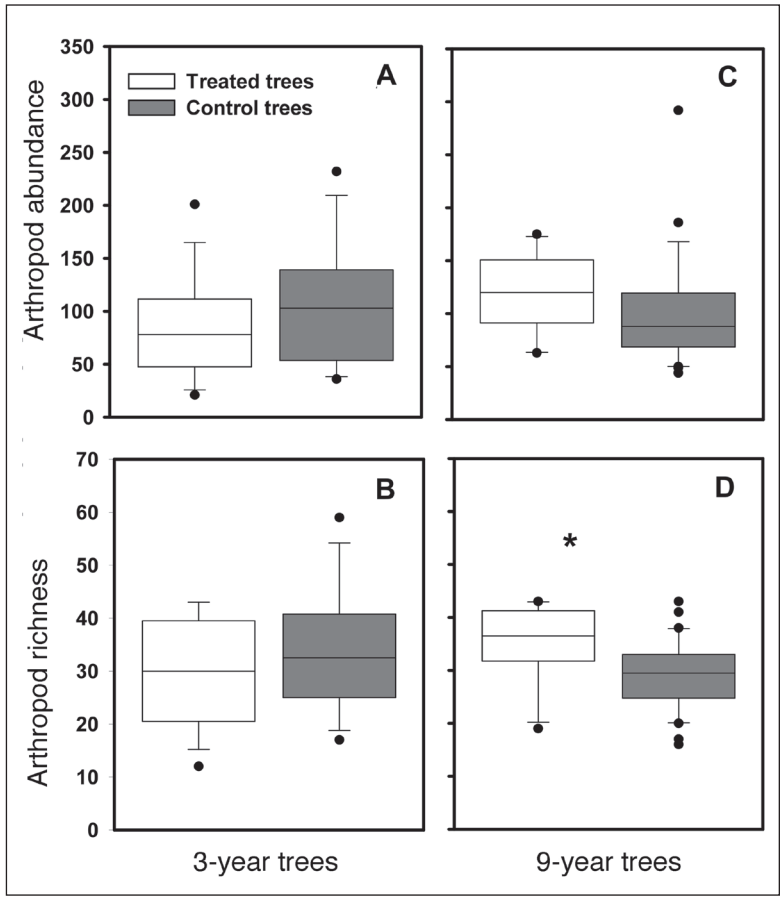

Figure 6. Box-and-whisker plots showing the median (dividing line in box), quartile (upper and lower ends of box), 10th and 90th percentiles (ends of lines), and outliers (dots) for: Arthropod abundance (top) and richness (bottom) in trees with and without detectable imidacloprid in trees treated 3-years (left) and 9-years (right) prior to sampling. Statistically significant differences are indicated by *.

Discussion

These data show that nearly a decade after the application of imidacloprid to Eastern Hemlock trees, the insecticide was still detectable among many of the treated trees, and that there was still a significant, but intermittent impact on HWA populations. The detection of a difference in HWA densitiy in February 2013 (nearly 11 years after its application), and the failure to detect a difference the previous year suggest that the concentrations of imidacloprid within the trees may be at the threshold of detectable efficacy.

The data also suggest that 9 years after application, the use of imidacloprid is associated with a change in species richness and community composition among canopy arthropods. The directionality of the shift in richness is, however, rather surprising with a statistically higher median alpha diversity associated both with treated trees as a group, and with those trees in which imidacloprid was found at detectable concentrations. The reasons for this pattern are not known, and merit further study.

In addition to shifts in median morphospecies richness, the 9-year group also provided indications of differences in community structure as shown by the ordination. However, those differences may be subtle, and based on the loss of statistical significance associated with the reduction in sample sizes, these shifts in community structure may also, at 9-years post-pesticide application, be approaching the limits of detection.

It is interesting to note that analysis by LC-MS/MS did not detect imidacloprid in 8 of the 9-year post-treatment trees, though there were differences in the composition of canopy-arthropod communities revealed by MRPP analysis. Additionally, several species may serve as indicators of imidacloprid treatment. In combination, these data raise the possibility that the sensitivity of the insect communities to imidacloprid in trees may exceed the sensitivity of the chemical analyses used to detect it. The data also suggest that there is a strong need to evaluate the long-term impacts of the use of imidacloprid on arthropod communities because this study found an unexpected increase in richness associated with imidacloprid-treated trees. Whether this pattern is generalizable and the ecological importance of these changes remain unknown.

In addition to the patterns found in the 9-year post-application trees, our data yielded a second surprise when analyses of the 3-year treated groups indicated differences in HWA density but not in community composition. A previous study by Dilling et al. (2009), in which arthropods were sampled approximately 1 year after imidacloprid application, found substantial impacts on both alpha diversity and community structure. We detected no differences only 3 years after application. Though interesting, our data have critical limitations, specifically the high potential for contamination by both imidacloprid and other insecticides within the control trees. The trees within the 3-year study group are arranged in a windbreak with 2–3 m separating each tree, a spacing which results in trees with interdigitated canopies, and a high potential for root grafting in this shallow-rooted species (Eckenwalder 2009, Fowells 1965, Frothingham 1915). The short distances also create a high

potential for lateral movement of imidacloprid through the soil (Felsot et al. 1998, Gupta et al. 2002). Both of these conditions could result in the presence of imidacloprid in varying concentrations within the control trees. It should also be noted that other insecticides were evaluated in the original study (e.g., dinotefuran); we did not test for the presence of these compounds and so their presence as a result of lateral contamination from nearby applications is unknown. Similarly, the study trees are located along the edge of an active experimental agricultural field used to grow products including fruit trees and corn, and contamination of our study trees from insecticide applications made to the adjacent field cannot be ruled out. We suggest that these factors may have influenced the lack of pattern in the canopy arthropod communities in our study trees.

Overall, our data indicate the use of imidacloprid for the control of HWA in Eastern Hemlock stands has the potential to provide protection from these invasive species for up to a decade, though it is worth noting that the HWA densities observed during this study were quite low, and the efficacy of the concentrations within the trees subject to high HWA pressures is not known. Although the duration of the impact may provide land-managers with options and flexibility regarding the timing and frequency of imidacloprid applications, our data also suggest that the legacy effects of the use of this systemic insecticide can be long-lasting for both the target species and non-target communities. Replication of this study is needed to further examine the stability, repeatability, and ecological meaning of the changes in arthropod richness and community composition found in Eastern Hemlocks nearly a decade after treatment with imidacloprid.

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