Received: 11 December 2023 | Accepted: 5 August 2024

DOI: 10.1111/afe.12647

ORIGINAL ARTICLE

Agricultural and Forest Entomology

The impact of imidacloprid on the subterranean survivorship of *Laricobius* (Coleoptera: Derodontidae), a biological control agent of *Adelges tsugae* (Hemiptera: Adelgidae)

Ashleigh P. Hillen[1] | Jeremiah R. Foley IV[2] | Aaron D. Gross[1] | Albert E. Mayfield III[3] | Jacob Williams[4] | Kang Xia[5] | Scott M. Salom[1]

[1]Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

[2]Department of Environmental Science and Forestry, Connecticut Agricultural Experiment Station, New Haven, Connecticut, USA

[3]Southern Research Station, USDA Forest Service, Asheville, North Carolina, USA

[4]Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

[5]School of Plant and Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

Correspondence

Ashleigh P. Hillen, Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, 1010 Lyle St, Radford, VA 24141, USA.
Email: ashleighh@vt.edu

Funding information

USDA Forest Service, Forest Health Protection (FHP), Grant/Award Number: 20-CA-11094200-126.

Abstract

1. The invasive hemlock woolly adelgid (HWA), *Adelges tsugae* (Annand) (Hemiptera: Adelgidae), has spread throughout most of the range of eastern hemlock, *Tsuga canadensis* (L.), and the entire range of Carolina hemlock, *Tsuga caroliniana* (Engelman), in the United States.

2. Integrated pest management (IPM) of HWA combines chemical applications with the release of biological control agents on untreated trees within the same stand. *Laricobius* spp., Rosenhauer (Coleoptera: Derodontidae), have been used as biological control agents of HWA since 2003 and have subterranean and arboreal life phases that are synchronous with HWA's lifecycle. When utilizing IPM tactics, there is potential for *Laricobius* spp. to settle below an insecticide-treated tree for its subterranean phase.

3. Field investigations assessed the impact of historical (five years post treatment in 2017) and recent imidacloprid soil treatments (via soil injection, soil drench, and tablet in November 2020) on the subterranean survivorship of *Laricobius* spp. by quantifying concentrations of imidacloprid and its metabolites to determine its potential impact on percent adult emergence from the soil.

4. We observed a significant treatment effect on mean soil concentration among application methods at the recent treatment site, but not the historical treatment site. Additionally, at the more recently treated site, significantly lower mean percent emergence was observed from soil drench and tablet imidacloprid applications after one year (2021), but by the following year (2022), that effect was no longer present.

5. This study supports recent recommendations to delay releases of *Laricobius* spp. for one-year post-treatment with imidacloprid. Furthermore, these data suggest when applying imidacloprid to a stand established with *Laricobius* spp., soil injection techniques pose the least risk to their subterranean survivorship.

KEYWORDS

biological control, Derodontidae, imidacloprid metabolites, IPM, toxicology

INTRODUCTION

In eastern North America, the non-native hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, was initially found in Richmond, VA in 1951 (Gouger, 1971; McClure, 1987) and is now present in 22 states of the eastern United States and two eastern Canadian provinces (Foley IV et al., 2021; Kantola et al., 2019). In its native range of western North America, China, Taiwan, Nepal and Japan (Havill et al., 2006), HWA coexists with *Tsuga* species without causing significant damage to its hosts. However, in its introduced range, HWA causes widespread mortality to eastern hemlock, *Tsuga canandensis* (L.) Carrière (Orwig et al., 2002), and Carolina hemlock, *T. caroliniana* Engelm. (Vose et al., 2013). HWA attaches itself to a host by inserting its stylet bundle at the base of needles. Saliva from the stylet bundle forms a salivary sheath, through which nutrients are obtained from the xylem ray parenchyma cells (Young et al., 1995). Feeding by HWA first induces premature needle drop and branch dieback, but as infestations progress over several years, an inability to produce new growth exacerbated by abiotic factors such as drought (Orwig et al., 2002) can lead to widespread tree mortality (McClure, 1991).

Biological control

Laricobius nigrinus, Fender, and *L. osakensis*, Montgomery and Shiyake, are two established introduced biological control agents of HWA in eastern North America (Foley IV et al., 2021; Zilahi-Balogh, Salom, & Kok, 2003). *Laricobius* spp. are well suited for predation of HWA, occupying two life stages (arboreal and subterranean) synchronous with the feeding and aestivation stages of the HWA sistens generation (Foley IV, McAvoy, Grubb, et al., 2022; Foley IV, McAvoy, Saint-Amant, et al., 2022; Zilahi-Balogh, Salom, & Kok, 2003). In the fall and winter, *Laricobius* spp. adults locate HWA-infested hemlocks, feed on developing nymphs and eventually oviposit in HWA ovisacs (Zilahi-Balogh, Salom, & Kok, 2003). In the spring, *Laricobius* spp. larvae feed on HWA eggs through the fourth larval instar, after which they drop below the tree's canopy, settle into the soil within the dripline and eclose to adulthood. Adults remain subterranean for a period of summer dormancy (aestivation) until seasonal cues induce emergence in the fall (September/October) (Hillen et al., 2023; Lamb et al., 2007; Zilahi-Balogh, Salom, & Kok, 2003).

Chemical control

Imidacloprid (IMI) is the most frequently used insecticide for the management of HWA (Coots et al., 2013; Crayton et al., 2020). A member of the Insecticide Resistance Action Committee (IRAC) subgroup 4 (neonicotinoids) (Sparks et al., 2021), IMI is a systemic insecticide that once ingested, and delivered to its target, functions as a competitive modulator of the nicotinic acetylcholine receptors within the central nervous system of insects (Fossen, 2006; Gervais et al., 2010). Acetylcholine is the major excitatory neurotransmitter within the central nervous system of insects (Grünewald & Siefert, 2019). Irreversible binding of IMI leads to a range of symptoms including overexcitement of the central nervous system, lethargy, paralysis and death (Mullins, 1993; Nauen & Bretschneider, 2002). In forestry and arboriculture, IMI is typically applied directly to trees via trunk injection and bark spray or to the soil through methods such as soil drench, soil injection or slow-release tablet formulations (Coots et al., 2013; Mayfield III et al., 2020; Steward et al., 1998).

Imidacloprid is relatively water soluble (octanol/water partition coefficient, $K_{ow} = 0.57$) (Gervais et al., 2010), and in treated hemlocks, it is translocated by the xylem throughout the tree and consumed by HWA via the xylem ray parenchyma cells, which they target for nutrient consumption (Coots et al., 2013; Steward et al., 1998; Young et al., 1995). Within hemlock trees, IMI first metabolizes into 5-hydroxy-imidacloprid (5H-IMI), followed by further metabolism into imidacloprid-olefin (IMI-OLE) (Nauen et al., 1998; Sur & Stork, 2003). However, soil composition (Cox et al., 1997), climatic and seasonal variations, tree diameter at breast height (DBH), internal water flow of treated trees (Ford et al., 2007), presence of surface water and chemical formulation all contribute to the varying rate at which IMI breaks down and which metabolites persist in the soil post-application (Fossen, 2006; Gervais et al., 2010; Wamhoff & Schneider, 1999).

Imidacloprid and its metabolites are known to persist in hemlock foliage 4–7 years post-treatment (Benton et al., 2015), and IMI itself has an approximate soil half-life of 62 days in the dark and <90 h when exposed to light (Cox et al., 2004). This persistence of IMI, 5H-IMI and IMI-OLE residues within hemlock tissue provides up to 5 years of protection against HWA after just one chemical treatment (Benton et al., 2015); however, persistence within soil has the potential to negatively impact the establishment of biological control agents. The metabolite IMI-OLE was found to be 16× more effective at the suppression of *A. gossypii*, Glover, and *Myzus persicae*, Sulzer, than its parent chemical, IMI (Nauen et al., 1998), and mortality of *Laricobius* spp. has been observed when beetles feed on HWA settled on foliage with IMI and IMI-OLE residues within the plant tissues (Eisenback, 2008). To our knowledge, the effect of soil applied IMI treatments on the subterranean survivorship of *Laricobius* spp. has not been studied to date.

Management

Current management recommendations for HWA incorporate an integrated pest management (IPM) approach that combines chemical applications with releases of biological control agents on untreated trees within the same hemlock stand (Mayfield III et al., 2020). Ideally, once predator populations have established, chemical residues in and around previously treated trees would persist at levels below which would be harmful to predators that naturally disperse to these trees. However, further dispersal of predators from release sites could inadvertently expose some individuals to chemical residues within hemlock tissues and soil at the base of treated trees (Eisenback et al., 2010; Mayfield III et al., 2020). These compounds have the potential to negatively impact populations of soil microarthropods; however, high variability in field settings which leads to inconsistent movement and adsorption of IMI (Knoepp et al., 2012) require variability in site selection for studying this phenomenon.

In the study reported here, we quantified the IMI, 5H-IMI and IMI-OLE chemical residues at three depths (0–1, 1–6, 6–11 cm) from soil treated with three different IMI application techniques; CoreTect™ tablets, soil drench and soil injection, to determine their direct impact on the survival of adult *Laricobius* spp. during their subterranean life phase. With the knowledge that *Laricobius* spp. burrow approximately 1–3 cm deep (in soils compacted to 0.5 and 0.2 kg/cm^2, respectively) (Hillen et al., 2023), the results from this study can help inform HWA managers on how and when to best apply IMI-based insecticides to soil where *Laricobius* spp. beetles are likely to become present.

MATERIALS AND METHODS

Field sites, insecticide treatments and *Laricobius* emergence traps

Two field sites in western Maryland (MD) and one in southwest Virginia (VA) were used for this study. Savage River State Forest (SRSF) in Garrett County, MD, was selected for its well documented historical treatment of hemlocks using IMI soil injection in 2017. Twenty trees were selected at SRSF—ten trees treated in 2017 with soil injection at one site (SRSF 2017) (39.651818, −79.098641) and ten untreated control trees at the other (SRSF Control) (39.62034, −79.144629). Because the 2017 soil injections were applied to all the trees in a localized stand (SRSF 2017), it was necessary to select the 10 control trees from a nearby, untreated stand (SRSF Control) with similar forest stand characteristics. Soils at the SRSF soil injection 2017 site were generally Gilpin channery silty loams with 10%–20% slopes, moderately eroded. Soils at the SRSF control site were generally Ungers, Calvin and Lehew channery loams, with 0%–10% slopes. The trees selected from the SRSF 2017 site were treated in 2017 using Merit® 75 Water Soluble Packets (WSP) (Bayer CropScience, Cary, NC, USA) suspended in Kioritz® (Kioritz Corporation, 7–2 Suehirocho 1-Chome, Ohme, Tokyo 198, Japan) and E-Z-ject™ Soil Injector Lite (ArborSystems, 10,168 L Street, Omaha, NE, USA) soil injectors. Imidacloprid application rates were based on the optimized dosage recommendations calculated using each tree's DBH (ranging from 15.5 to 36.6 cm) and the number of fluid millilitres of active ingredient applied to the soil in each injector pump (Benton et al., 2016; Benton & Cowles, 2017).

Kentland Farm (KF) (37.207735, −80.589635) in Blacksburg, VA is home to a 20-year-old eastern hemlock plantation and field insectary on a 0.4 ha plot of previously used pastureland. This site was selected for the study examining effects of recent IMI treatments. This site has a history of fertilization, HWA infestation and biological control releases, but no previous insecticide treatments (McAvoy et al., 2017). Soils at KF were generally Guernsey silty loam with 2%–7% slopes (Soil Survey Staff, 2023).

At KF, a completely randomized block design consisting of five blocks, each with four treatments was installed using 20 eastern hemlocks within the hemlock plantation. The KF site was first cleared of extraneous understory debris and then treated with IMI on 17 November 2020, using label instructions and optimal IMI dose recommendations for HWA control on hemlock trees based on each tree's DBH (Benton & Cowles, 2017). Imidacloprid treatments included (1) soil drench, (2) soil injection, (3) CoreTect™ tablets (Bayer CropScience, Cary, NC, USA) and (4) untreated control. CoreTect tablets were placed 5–12 cm below the soil surface, approximately 46 cm from the base and evenly spaced around the trunk. For the soil injection and soil drench applications, two Merit® 75 WSP (Bayer CropScience, Cary, NC, USA) were mixed into one container to a final volume of 2.01 L. Half was measured into individual pre-calculated volumes for the soil drench application which was applied to five trees (one tree per block) using the method described by Benton and Cowles (2017). The other half was poured into a Kioritz® injector and used to apply IMI to the soil at the base of five trees (one per block) at a depth of up 7.6–20.0 cm (Steward et al., 1998). A total of 48 CoreTect tablets and 1.4 L of WSP solution was used, resulting in 69.9 g of active ingredient for the entire 0.4 ha plot (Bayer CropScience, Cary, NC, USA) (Benton & Cowles, 2017).

Four PVC emergence traps internally lined with 7.6 cm yellow sticky cards (Olson Products Inc., Medina, OH) (Foley IV, McAvoy, Grubb, et al., 2022) were placed approximately 46 cm from the base of every study tree (one in each cardinal direction) in February 2021. In 2022, an additional fifth trap was added to each study tree (at one randomly chosen intercardinal direction) to account for any naturally occurring *Laricobius* spp. emergence not resulting from experimental larval release. *Laricobius* spp. larvae used in this study were obtained in April 2021 and 2022 by field-collection of foliage, previously known to have established populations of *Laricobius* spp. in Blacksburg and Radford, VA (Foley IV et al., 2019; McAvoy et al., 2020). Individual *Laricobius* spp. used in this study were not identified to species; however, wild populations in the New River Valley, which includes Blacksburg, VA have been documented to contain mostly (up to 98%) *L. nigrinus* (Foley IV et al., 2019). To collect pre-pupal larvae, HWA-infested foliage was clipped into 31 cm segments and inserted into hydrated OASIS® blocks wrapped in Parafilm™ M (Fisher Scientific, Hampton NH) to retain moisture. These foliage bouquets were held in 18.9-L buckets, stored outdoors and were checked daily. Once matured to the fourth instar, larvae dropped from foliage and were collected using a clean, moistened paintbrush and transferred to petri dishes containing moistened filter paper for field transport.

Ten larvae were placed into each trap in 2021 (3 months post-treatment) and in 2022 (15 months post-treatment), except for the fifth trap (field control) in 2022, totalling 1600 larvae per year. At both SRSF 2017 and SRSF Control, 10 pre-pupal larvae were placed into each trap within 48 h of dropping from foliage during a 2-day period from 21 to 22 April 2021 and 20 to 21 April 2022. At KF, 10 pre-pupal larvae were placed into each trap during a 4-day period from 14 to 18 April 2021 and 13 to 17 April 2022 within 24 h of dropping from foliage. Traps were monitored monthly at each field site from April to December, where they were checked for disturbance and early emergence. In December, yellow sticky cards were removed from each trap and then examined to record percent emergence. Soil moisture readings in volumetric water content (VWC) were collected monthly using a Hydrosense II Handheld Soil Moisture Sensor (CS659 12-cm) (Campbell Scientific, Australia).

Soil insecticide residue analysis

To detect insecticide residue concentrations, soil samples were collected at KF in November 2020 to establish a pre-treatment baseline, and again at KF and SRSF in April 2021 and 2022 at the time of larval placement. Pre-treatment soil samples were not possible at SRSF, due to treatments taking place in 2017. Soil samples were collected using a 91.4-cm One-Piece Soil Sampler (Global Gilson, Lewis Center, OH) (22.9 × 1.9 cm diameter sample size). Soil samples were contained in plastic bags and held on ice packs for transit until immediately frozen at −20°C once returned from the field. Samples consisted of the aggregate soil at each cardinal direction, 46 cm from the base of the tree, at depths of 0–1, 1–6 and 6–11 cm.

Soil samples were held at −20°C before being freeze-dried and stored at −20°C until analysed for IMI, 5H-IMI and IMI-OLE, using liquid chromatography tandem mass spectrometry (LC/MS/MS). About 1 g of freeze-dried soil sample was weighed and exact weight was recorded before being transferred into a 35 mL tube (DWK Life Sciences, Rockwood, TN, P/N 05–558-12A). The sample was spiked with 20 μL mixture of IMI-d4 (Toronto Research Chemicals, Toronto, ON, Canada, CAS# 1015855–75-0) IMI-OLE-d3 (Cambridge Isotope Laboratories, Laboratories, Tewksbury, MA, CAS# 2483830–31-3) and 5H-IMI-d4 (Clearsynth Canada, Mississauga, ON, Canada, CAS# 380912–09-4), each at 500 ppb, as internal standards for the target analytes to account for the loss of the target analytes during sample extraction, cleanup and analysis. The sample was then mixed with ∼0.5 g $MgSO_4$ (Fisher Chemical, P/N MG65-500) and ∼0.5 g NaCl (Thermo Fisher Scientific, Waltham, MA, P/N BP358-1), and 10 mL HPLC grade acetonitrile (Thermo Fisher Scientific, P/N A955-4), mixed using a VWR® Mini Vortexer at the highest speed for 2 min, and then centrifuged at 3041 g for 10 min. As much supernatant as possible was transferred, without disturbing the sediment, into another test tube containing ∼0.1 g C18 Endcapped SPE Bulk Sorbant (Agilent Technologies, Santa Clara, CA, P/N 5982–1382) and ∼0.05 g Carbon SPE Bulk Sorbant (Agilent Technologies, P/N 5982–4482) for further cleanup. The mixture was first vortexed at speed 7–8 for 1.5 min and then centrifuged at 3041 g for 10 min. Five millilitres supernatant was transferred, without disturbing the sediment, to a 6 mL test tube (Thermo Fisher Scientific, P/N 14–961-32) and dried using an Automated Evaporation System-LV TurboVap® at 75°C under 10 psi of nitrogen for 25 min. The dried residue was brought to room temperature and reconstituted with 1.0 mL of 3:7 mobile phase mixture (3 parts 5 mM NH_4AC in MeOH:7 parts 5 mM NH_4AC in H_2O) before being filtered through a 0.2 μm PVDF filter (Thermo Fisher Scientific, P/N F2513-4) into a 2 mL amber glass (HPLC vial Agilent Technologies, P/N 5182–0716). The filtered samples were either immediately analysed on the LC/MS/MS (Agilent 1290 Infinity HPLC/ Agilent 6490 Triple Quad LC–MS) or stored frozen (−20°C) and brought to room temperature before being analysed.

A seven-point linear standard curve was constructed for each target compound using the peak area ratio for a compound and its stable isotope against the concentrations of the seven standards of the target compounds. The concentrations of the three target analytes were determined using the seven-point linear standard curve against the peak area ratio for a compound and its stable isotope in a tested soil sample. Although the stable isotopes of the target analytes were used as internal standards to correct the loss of the target analytes during sample extraction, cleanup and instrumental analysis, 100% spike recovery rate is often unachievable due to various interactions with complex soil components and instrument matrix effect. For this study, the recovery rates of the three target analytes were 85%–95% for the spiked soil samples, which were well within the Environmental Protection Agency's (EPA's) 70%–110% spike recovery rate range required for pesticides analysis (EPA, 2007). The spiked sample recovery rate for a target analyte is reported to indicate a methods' validity; however, it is not normally used to adjust the reported concentration value of a target analyte in a sample. The method detection limits were 0.167, 0.056 and 0.167 ppb (μg/kg dry soil basis) for IMI, 5H-IMI and IMI-OLE, respectively.

Statistical analysis

Beetle percent emergence and chemical quantity data were collected over a two-year period (2021–2022) from both the KF and SRSF field sites. Percent emergence and chemical quantities were measured at the tree level and all analyses were conducted using R (R Core Team, 2021) using the packages car, lme4, lmerTest and multcomp.

Beetle emergence percentage was calculated as the number of adults emerged divided by the number of larvae (10) placed into the soil beneath each trap, expressed as a percent. In 2022, an extra trap was placed at each tree without larval release, to quantify the number of beetles emerging from naturally occurring *Laricobius* populations. In these 2022 control traps, no beetles emerged at SRSF, and only three beetles emerged from the 20 control traps at KF (emergence rate = 0.015). A one sample *t*-test showed that naturally occurring emergence was not significantly different from zero ($p = 0.1649$), so no adjustment to the other trap emergence counts was needed. The analysis of all other emergence traps followed the methodology of Foley IV, McAvoy, Grubb, et al., 2022, where emergence was modelled as a generalized linear mixed model (GLMM), using the percent emergence as a response with independent variables including treatment, year and their interaction. Ten larvae were placed in each trap, therefore an offset of 10 was applied to the GLMM. The study design

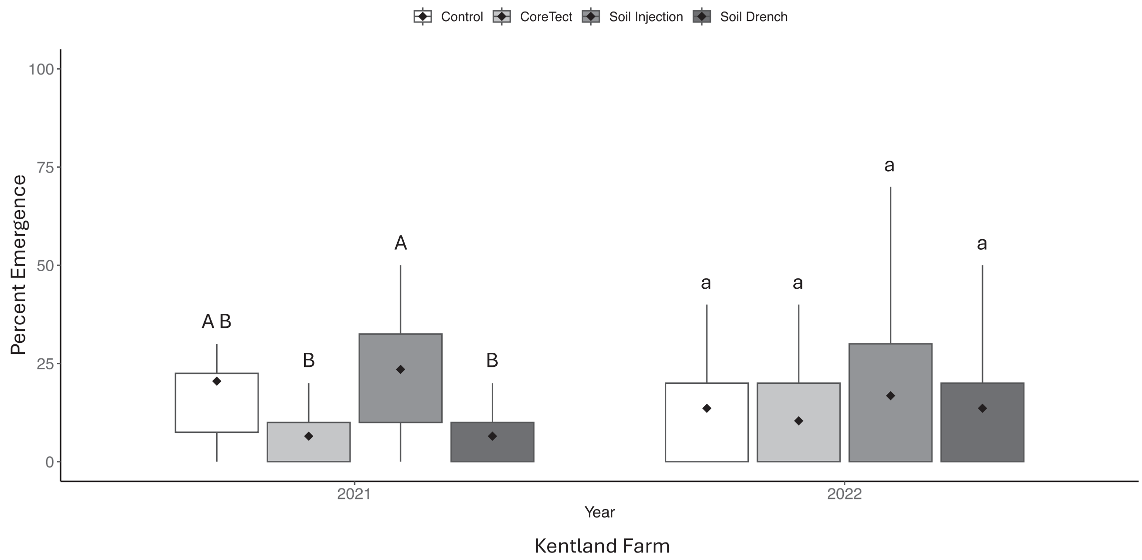

FIGURE 1 Box plot depicting the percent emergence of adult *Laricobius* spp. for each treatment (control, soil injection, CoreTect and soil drench) at Kentland Farm (KF) in 2021 (1-year post-treatment) and 2022 (2 years post-treatment). Different colour boxes represent each treatment, diamonds in each box represent the means and when present, vertical lines extend to the maximum and minimum observed percent emergence. Boxes labelled with the same letter (capital letters for 2021 and lowercase letters for 2022) do not differ by treatment within each year. A pairwise *t*-test was used for mean comparison. Raw data were transformed by square root (Emergence +0.5) for statistical analysis but means of untransformed data are shown.

differs between the KF and the SRSF field sites, so the random block effect used in the GLMM for each site is different. For KF, the repeated tree measurements are nested in the overall block effect. For SRSF, the repeated tree measurements were accounted using a block random effect. The model for each site used a type II ANOVA from the car package and the multcomp package to investigate the multiple comparisons of treatment effects conditional on year.

The coefficients for the categorical variables treatment and year are relative to a baseline level present in the model when that level is observed. The baseline levels to which the coefficients are compared is the control treatment and the year 2021. The coefficients are explaining a log scaled emergence rate; therefore, the baseline predicted percent emergence for year 2021 and the control treatment is the exponentiated intercept coefficient.

Concentrations of IMI, 5H-IMI and IMI-OLE were measured in parts per billion at three depths for each tree in both 2021 and 2022. To model the concentrations of each of these chemicals, a linear mixed effect regression model was used with an identical random effect structure as described above for the percent emergence analysis for each site. To account for the assumption of normality, a logarithmic transformation was used with the chemical concentrations plus one, as there were exact zeros in the observed concentrations. Independent variables for this analysis included year, depth, treatment and all higher order interactions of these three main effects. Models were fit using the lmerTest package, with a type II ANOVA from the car package and a Satterthwaite correction. Like the emergence analysis, the multiple comparisons of treatment effects conditional on year were analysed using the multcomp package.

RESULTS

Laricobius emergence

At KF in 2021, the CoreTect and soil drench treatments had a significantly ($p < 0.05$) lower mean percent emergence than the soil injection treatment (Figure 1). In 2021, percent emergence in the control did not differ from any of the insecticide treatments (Figure 1). In 2022, no significant differences among treatments on the mean percent emergence was found at KF (Figure 1). At SRSF over both years of this study (2021 and 2022), there was no significant difference in the mean percent emergence between the control and soil injection 2017 treatments (Figure 2).

Soil insecticide residue analysis

Based on the results of the Type II ANOVA, an overall significant treatment effect was found at KF on the concentrations of IMI ($F = 49.75$, df $= 3$, $p < 0.001$), 5H-IMI ($F = 15.41$, df $= 3$, $p < 0.001$) and IMI-OLE ($F = 28.68$, df $= 3$, $p < 0.001$) in the soil. Year had a significant effect ($F = 5.3$, df $= 1$, $p < 0.05$) on the concentration of 5H-IMI found at KF. In addition, there was a significant interaction between treatment and year ($F = 2.95$, df $= 3$, $p < 0.05$) at KF on the concentration of 5H-IMI in the soil. At SRSF, the overall concentration of IMI was significantly affected by treatment ($F = 14.03$. df $= 1$, $p < 0.01$), year ($F = 18.32$, df $= 1$, $p < 0.001$) and depth ($F = 12.42$, df $= 2$, $p < 0.001$), but not a three-way interaction. The results are depicted in figures rather than in table form due to the specific

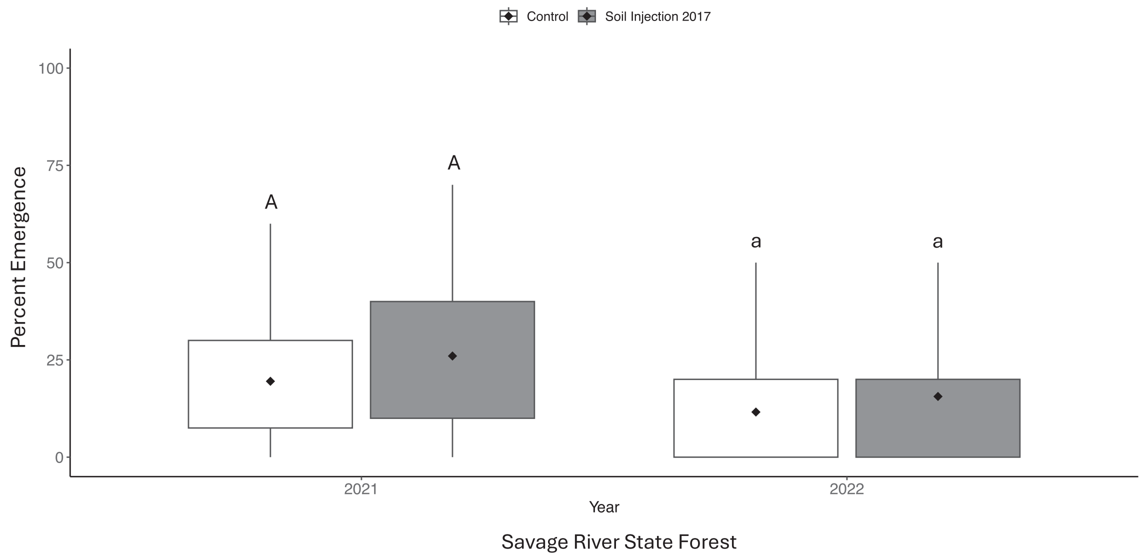

FIGURE 2 Box plot depicting the percent emergence of adult *Laricobius* spp. for each treatment (control and soil injection 2017) at Savage River State Forest (SRSF) in 2021 (1-year post-treatment) and 2022 (2 years post-treatment). Different colour boxes represent each treatment, diamonds in each box represent the means and when present, vertical lines extend to the maximum and minimum observed percent emergence. Boxes labelled with the same letter (capital letters for 2021 and lowercase letters for 2022) do not differ by treatment within each year. A pairwise *t*-test was used for mean comparison. Raw data were transformed by square root (Emergence +0.5) for statistical analysis but means of untransformed data are shown.

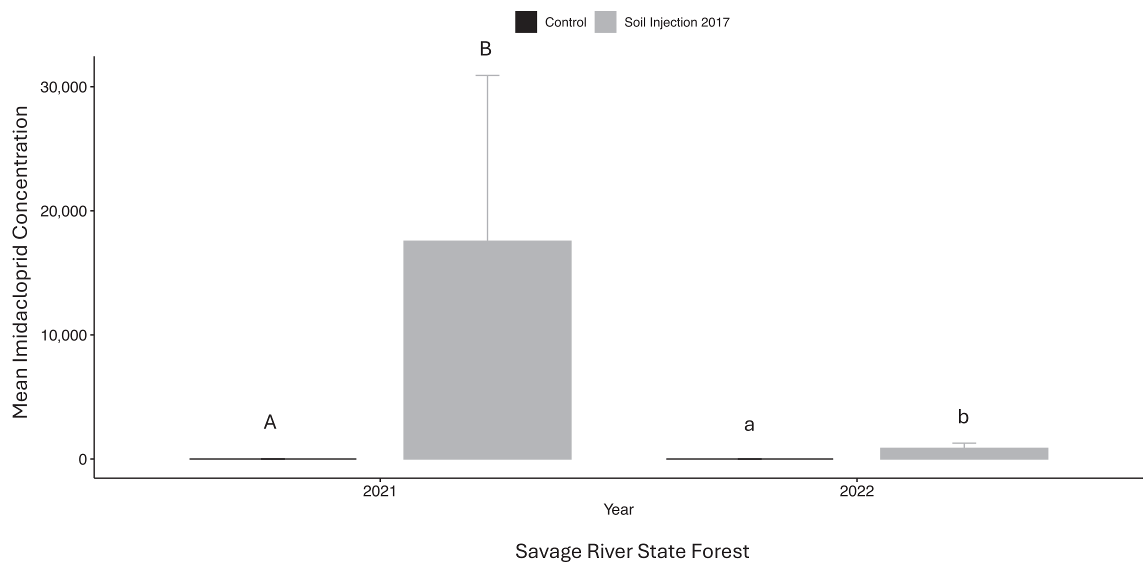

FIGURE 3 Bar plot depicting the mean (+ SE) imidacloprid (IMI) concentration in parts per billion (ppb) for each treatment (control and soil injection 2017) at Savage River State Forest (SRSF) in 2021 (4 years post-treatment) and 2022 (5 years post-treatment). The height of the bar represents the mean and vertical lines represent the standard error. Means labelled with the same letter (capital letters for 2021 and lowercase letters for 2022) do not differ by treatment within each year. A pairwise *t*-test was used for mean comparison. Raw data were transformed by log ($x + 1$) for statistical analysis but means of untransformed data are shown.

significant interactions. A significant interaction at SRSF was present between the concentration of IMI in the soil between treatment and year ($F = 18.71$, df $= 1$, $p < 0.001$) (Figure 3) and treatment and depth ($F = 11.12$, df $= 2$, $p < 0.001$) (Figure 4).

In 2021 at KF, the soil drench treatment resulted in a significantly higher ($p < 0.05$) mean concentration of IMI than CoreTect and control treatments at the 0–1 and 1–6 cm depths (Table 1). In 2022, the soil drench treatment resulted in higher concentrations of IMI than the soil injection, CoreTect and control treatments at the 0–1 and 1–6 cm depths (Table 1). The soil drench treatment also resulted in significantly higher ($p < 0.05$) mean concentration of IMI-OLE than the other three treatments at the 0–1 cm depth in 2021 and at both

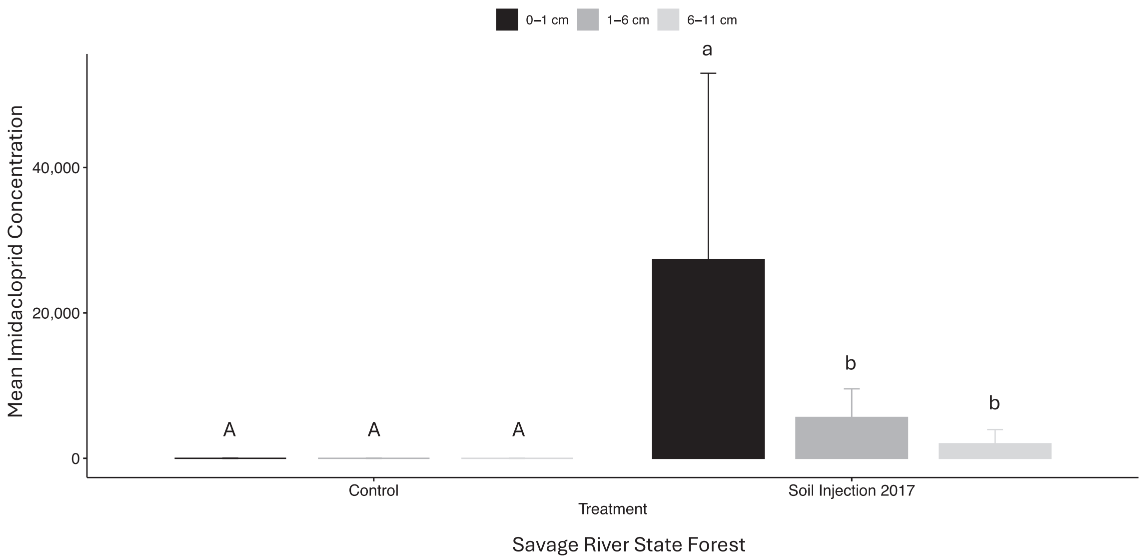

FIGURE 4 Bar plot depicting the mean (+ SE) imidacloprid (IMI) concentration in parts per billion (ppb) for each depth; 0–1, 1–6 and 6–11 cm at Savage River State Forest (SRSF) for both treatments (control and soil injection 2017) for both 2021 (4 years post-treatment) and 2022 (5 years post-treatment). The height of the bar represents the mean and vertical lines represent the standard error. Means labelled with the same letter (capital letters for control and lowercase letters for soil injection 2017) do not differ by depth within each treatment. A pairwise *t*-test was used for mean comparison. Raw data were transformed by log (*x* + 1) for statistical analysis but means of untransformed data are shown.

TABLE 1 Mean (SE) chemical residues (ppb, μg/kg dry soil basis) of imidacloprid (IMI), imidacloprid-olefin (IMI-OLE) and 5-hydroxy-imidacloprid (5H-IMI) at Kentland Farm (KF) by treatment, soil depth and year.

			Treatment			
Chemical	Year	Depth (cm)	Soil drench	Soil injection	CoreTect	Control
IMI	2021	0–1	4168.8 (1154.7)^{A,a}	1653.4 (698.9)^{AB,a}	224.3 (146.0)^{B,a}	2.0 (2.0)^{B,a}
	2021	1–6	3620.6 (1293.5)^{A,a}	795.4 (687.5)^{AB,a}	57.5 (52.0)^{B,a}	0.9 (0.9)^{B,a}
	2021	6–11	2481.4 (1167.0)^{A,a}	5303.1 (4360.9)^{A,a}	2.8 (1.9)^{A,a}	2.6 (1.3)^{A,a}
	2022	0–1	4167.9 (1171.2)^{A,a}	348.1 (225.2)^{B,a}	4.4 (1.4)^{B,a}	1.1 (0.7)^{B,a}
	2022	1–6	2520.1 (476.0)^{A,a}	889.5 (520.1)^{B,a}	246.6 (169.4)^{B,a}	0 (0)^{B,a}
	2022	6–11	1760.0 (557.7)^{A,a}	1427.5 (759.9)^{A,a}	1936.9 (1845.4)^{A,a}	0.2 (0.2)^{A,a}
IMI-OLE	2021	0–1	45.1 (5.7)^{A,a}	19.4 (8.6)^{B,a}	2.0 (1.6)^{B,a}	0 (0)^{B,a}
	2021	1–6	104.3 (36.0)^{A,a}	37.5 (24.2)^{AB,a}	0.8 (0.8)^{B,a}	0 (0)^{B,a}
	2021	6–11	33.3 (9.5)^{AB,a}	42.4 (17.4)^{A,a}	0 (0)^{B,a}	0 (0)^{B,a}
	2022	0–1	54.4 (13.3)^{A,a}	7.5 (5.5)^{B,a}	0 (0)^{B,a}	0 (0)^{B,a}
	2022	1–6	46.3 (9.4)^{A,a}	9.1 (5.4)^{B,a}	3.5 (2.6)^{B,a}	0 (0)^{B,a}
	2022	6–11	21.3 (6.2)^{A,a}	13.4 (8.2)^{A,a}	21.4 (19.7)^{A,a}	0 (0)^{A,a}
5H-IMI	2021	0–1	10.2 (3.9) ^{A,a}	5.3 (2.5)^{AB,a}	0.8 (0.8)^{AB,a}	0 (0)^{B,a}
	2021	1–6	8.7 (4.3) ^{A,a}	19.3 (17.0)^{A,a}	0.1 (0.1)^{A,a}	0 (0)^{A,a}
	2021	6–11	6.1 (2.8)^{A,a}	11.7 (6.5)^{A,a}	0 (0)^{A,a}	0 (0)^{A,a}
	2022	0–1	54.6 (17.7)^{A,a}	6.5 (4.6)^{B,a}	0 (0)^{B,a}	0 (0)^{B,a}
	2022	1–6	35.6 (8.0)^{A,a}	11.1 (7.0)^{AB,a}	1.8 (1.8)^{B,a}	0 (0)^{B,a}
	2022	6–11	20.5 (7.1)^{A,a}	18.5 (13.6)^{A,a}	66.2 (65.2)^{A,a}	0 (0)^{A,a}

Note: For a given chemical, treatment and year, means within the same row (depth) labelled with the same uppercase letter are not significantly different by treatment ($\alpha = 0.05$). For a given chemical, treatment and year, means within the same column (treatment) labelled with the same lowercase letter are not significantly different by depth ($\alpha = 0.05$). Raw data were transformed by log ($x + 1$) for statistical analysis but means of untransformed data are shown.

the 0–1 and 1–6 cm depths in 2022 (Table 1). The concentration of 5H-IMI in 2021 at 0–1 cm was significantly higher ($p < 0.05$) from the soil drench, soil injection and CoreTect treatments when compared with the control and in 2022, the soil drench treatment has significantly higher ($p < 0.05$) concentration of 5H-IMI than the other three treatments at 0–1 cm (Table 1). While significant ($p < 0.05$) differences between treatments were present in the mean soil concentration at a given depth at KF, depth alone did not have a significant effect on the mean concentration of soil residues within each treatment for a given chemical and year (Table 1).

Soil moisture data

Soil moisture data were collected at all three field sites between 2021 and 2022, during study months (April–December). Over both field seasons, the mean soil moisture at SRSF 2017 treatment field site was 16.7% with a mean minimum of 10.0%. At the SRSF Control site the mean moisture content was 20.7% with a minimum of 14.2%. The overall mean soil moisture at KF between 2021 and 2022 was 16.3% with a mean minimum of 10.9%.

DISCUSSION

This is the first study, to the best of our knowledge, to document the treatment effect of IMI soil applications on the subterranean survivorship of *Laricobius* spp. In addition, this study reported the different treatment effects on mean concentrations of IMI, 5H-IMI and IMI-OLE at different depths within the soil profile. The use of the historically treated site in western MD allowed us to take advantage of operational activities to study potential effects on subterranean survivorship and mean metabolite concentrations 5 years post-treatment. The findings from this study show that IMI, 5H-IMI and IMI-OLE were still present in soils 5 years post-treatment from soil injection treatments. Previous studies found that IMI and IMI-OLE were present in foliage 4–7 years post-treatment with IMI (Benton et al., 2015), IMI present in soil one-year post-treatment (Knoepp et al., 2012) and in surface waters downstream from treatment sites 1-year post-treatment (Benton et al., 2016). At the site where all three application methods were compared, the soil drench treatment resulted in the highest concentration of IMI, 5H-IMI and IMI-OLE in the soil compared to CoreTect 5 months post-treatment and compared to both Coretect and soil injection 17 months post-treatment.

During the first year of this study (2021) at KF, the soil drench and CoreTect treatments had the lowest mean percent emergence, but that effect was no longer present in 2022 (Figure 1). In 2021, both the soil drench and soil injection treatments resulted in significantly higher mean soil concentrations of IMI at the 0–1 and 1–6 cm depths and IMI-OLE at the 1–6 cm depth (Table 1). Interestingly, a significantly higher mean percent emergence occurred in the soil injection treatment in 2021 than both the soil drench and CoreTect treatments (Figure 1). In 2022, the soil drench treatment had significantly higher mean concentrations of IMI, IMI-OLE and 5H-IMI at the 0–1 cm depths and IMI and IMI-OLE at the 1–6 cm depths (Table 1), but no significant differences in emergence percentage was found between treatments (Figure 1). These data suggest that mean percent emergence was affected by the soil drench and CoreTect tablet application methods in the first year of the study, but not in the second year. This further supports the timing guidelines for releasing *Laricobius* spp. 1 year after application with IMI as suggested in Mayfield III et al. (2020). The significantly higher mean percent emergence from the soil injection treatment at KF during the first year of this study (Figure 1) suggests that although significant concentrations of IMI and IMI-OLE remained in the soil from this technique in 2021 (Table 1), it posed less risk to the subterranean survivorship of *Laricobius* spp. than soil drench or CoreTect tablet application techniques in the first year of this study.

The different treatment effects on percent emergence could be attributed to the depth at which each application technique is applied, the burrowing depth of *Laricobius* spp., and the bioavailability of the compound itself, which can vary greatly depending on soil composition and time (Oi, 1999). Soil drench applications are typically applied directly to the soil surface. In contrast, CoreTect tablets are buried 5–12 cm below the soil surface, and soil injections apply the chemical in suspension at a depth of 7.6–20.0 cm (Benton et al., 2015; Steward et al., 1998). Maximum *Laricobius* spp. larval burrowing depth has been demonstrated to be about 3 cm (Hillen et al., 2023), so soil injection and Coretect applications direct the chemical below the depth where *Laricobius* typically aestivates. The higher percent *Laricobius* spp. emergence observed from the soil injection treatment 1 year after treatment (2021, Figure 1) may have been due to reduced insect exposure to IMI, because the chemical was injected well below the larval burrowing zone. It is important to consider the lethal dose of IMI for *Laricobius* spp.; 2400 ppb with a 6-day LD_{50} value of 1.8 ng IMI per individual (Eisenback et al., 2010) and that each reported soil concentration value (Table 1) is a mean concentration (SE) reported from the aggregation of four soil samples per depth, per tree. This study attempted to create the 'worst case scenario' by placing the emergence traps at the same distance from the tree that the IMI was applied to expose the larvae to as much IMI, IMI-OLE and 5H-IMI as possible. An individual *Laricobius* spp. would not necessarily be exposed to that exact reported mean concentration nor would that entire concentration be bioavailable to that individual given the variability of soil organic matter, water flow and direct exposure within the soil (Oi, 1999). Furthermore, in an IPM field scenario, *Laricobius* spp. larvae drop from a tree's dripline to the soil, which includes a much larger area and would decrease the likelihood that an individual larvae would land exactly where IMI was injected, drenched or applied using a tablet.

Additional factors that could have impacted the total emergence of *Laricobius* spp. at all sites include abiotic conditions, transportation and handling of larvae before placement into emergence traps, and trap disturbance by animals throughout the duration of the experiment (Foley IV, McAvoy, Grubb, et al., 2022; Zilahi-Balogh, Humble, et al., 2003). The relatively low emergence rates described in this experiment are standard for *Laricobius* spp. and align with other studies in both the lab (37.5 ± 13.6%) and field sites in southwest Virginia (17.1 ± 0.8%) (Foley IV et al., 2021; Foley IV, McAvoy, Grubb, et al., 2022). Abiotic factors such as soil moisture in the field (Foley IV, McAvoy, Grubb, et al., 2022) and lab (Lamb et al., 2007) have been shown to significantly impact the subterranean survivorship of *Laricobius* spp. Soil moisture ranging between 20% and 40% have been identified as the ideal conditions for success of aestivation in *Laricobius* spp. (Foley IV et al., 2021; Lamb et al., 2007). Based on the relatively low mean minimum soil moisture content across all three sites and the known ideal conditions for *Laricobius* spp., soil moisture levels could have negatively affected the overall percent emergence data collected from this study.

While numerically different mean IMI, 5H-IMI and IMI-OLE concentrations existed between the SRSF 2017 and SRSF control site from 2021 to 2022 (Figure 3), there was high variation in mean ranges as well as a great number of non-detectable levels of residues within those soil samples. Non-detectable levels of residues were functionally analysed as zeros, and the highest metabolite concentrations were not excluded as outliers, which contributed to the lack of significant differences between groups. The wide range in mean chemical residue concentration present in the soil at the SRSF 2017 site demonstrates that IMI, 5H-IMI and IMI-OLE residues can persist in the soil below IMI treated trees 5 years post-treatment using soil injection techniques.

CONCLUSIONS

This study supports previous recommendations (Mayfield III et al., 2020) to delay releases of *Laricobius* spp. for 1 year post-treatment with IMI. Based on the mean percent emergence data from KF, the significant treatment effect from soil drench and CoreTect tablet application techniques ($p < 0.05$) in year one (2021) was no longer observed by year two (2022). Additionally, although IMI, IMI-OLE and 5H-IMI were all present at low concentrations 5 years post-treatment with soil injection at SRSF, no evidence existed that these residues affected mean percent emergence of the beetles. Lastly, these data suggest when applying IMI to a stand already established with *Laricobius* spp., soil injection techniques pose the least risk to their subterranean survivorship due to the significantly higher mean percent emergence ($p < 0.05$) from the soil injection treatment at KF during the first year of this study.

AUTHOR CONTRIBUTIONS

Ashleigh P. Hillen: Conceptualization; formal analysis; investigation; methodology; writing – original draft; writing – review and editing. **Jeremiah R. Foley IV:** Conceptualization; data curation; formal analysis; methodology; writing – original draft; writing – review and editing. **Aaron D. Gross:** Conceptualization; formal analysis; methodology; validation; writing – review and editing. **Albert E. Mayfield III:** Conceptualization; formal analysis; investigation; methodology; resources; validation; visualization; writing – original draft; writing – review and editing. **Jacob Williams:** Data curation; formal analysis; software; validation; writing – original draft; writing – review and editing. **Kang Xia:** Conceptualization; data curation; formal analysis; methodology; resources; supervision; validation; writing – review and editing. **Scott M. Salom:** Conceptualization; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; writing – original draft; writing – review and editing.

ACKNOWLEDGEMENTS

We thank Ary James, Carrie Preston, Rachel Hoffman, Ryan Mays, Olivia Andrews and Tom McAvoy for their assistance in the field. Carrie Jubb, Holly Gatton, Dr. Sheldon Hillaire and Dr. Aihua Wang for their training and technical assistance in the lab. Patrick Simmons and Biff Thompson with the Maryland Department of Natural Resources. We would also like to thank Dr. Elizabeth McCarty for her consultation and experience during the planning and analysis of this study. This research was funded by the USDA Forest Service, Forest Health Protection (FHP) grant 20-CA-11094200-126.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Ashleigh P. Hillen https://orcid.org/0000-0001-7423-123X

REFERENCES

Benton, E., Grant, J.F., Cowles, R., Webster, J., Nichols, R., Lagalante, A. et al. (2016) Assessing relationships between tree diameter and long-term persistence of imidacloprid and olefin to optimize imidacloprid treatments on eastern hemlock. *Forest Ecology and Management*, 370, 12–21.

Benton, E.P. & Cowles, R.S. (2017) *Optimized insecticide dosage for hemlock woolly adelgid control in hemlock trees*. Tifton, Georgia: The University of Georgia Warnell School of Forestry and Natural Resources. WSFNR-17-01. Available at: https://beershebaspringshistoricalsociety.org/hemlocks/Benton%20Chemical%20Treatment%20Guideline.pdf [Accessed 17th Nov 2020].

Benton, E.P., Grant, J.F., Webster, R.J., Nichols, R.J., Cowles, R.S., Lagalante, A.F. et al. (2015) 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. *Journal of Economic Entomology*, 108(6), 2672–2682.

Coots, C., Lambdin, P., Grant, J. & Rhea, R. (2013) Spatial and temporal distribution of residues of imidacloprid and its insecticidal 5-hydroxy and olefin and metabolites in eastern hemlock (Pinales: Pinaceae) in the southern Appalachians. *Journal of Economic Entomology*, 106(6), 399–406.

Cox, L., Hermosín, M. C., Celis, R. & Cornejo, J. (1997) Sorption of two polar herbicides in soils and soil clays suspensions. *Water Research*, 31(6), 1309–1316. https://doi.org/10.1016/s0043-1354(96)00337-5

Cox, L., Hermosín, M.C. & Cornejo, J. (2004) Influence of organic amendments on sorption and dissipation of imidacloprid in soil. *International Journal of Environmental Analytical Chemistry*, 84(1–3), 95–102.

Crayton, S.M., Wood, P.B., Brown, D.J., Millikin, A.R., McManus, T.J., Simpson, T.J. et al. (2020) Bioaccumulation of the pesticide imidacloprid in stream organisms and sublethal effects on salamanders. *Global Ecology and Conservation*, 24, e01292.

Eisenback, B.M. (2008) *Prey-mediated effects of imidacloprid on Laricobius nigrinus (Coleoptera: Derodontidae) and Sasajiscymnus tsugae (Coleoptera: Coccinellidae), two predators of hemlock woolly adelgid*. Blacksburg: Virginia Polytechnic Institute and State University. PhD Dissertation. Available at: https://vtechworks.lib.vt.edu/bitstream/handle/10919/28253/Eisenback_ETD.pdf?sequence=1&isAllowed=y [Accessed 18th March 2023].

Eisenback, B.M., Salom, S.M., Kok, L.T. & Lagalante, A.F. (2010) Lethal and sublethal effects of imidacloprid on hemlock woolly adelgid (Hemiptera: Adelgidae) and two introduced predator species. *Journal of Economic Entomology*, 103(4), 1222–1234.

Environmental Protection Agency (EPA). (2007) *Method 1699: pesticides in water, soil, sediment, biosolids, and tissue by HRGS/HRMS*. United States Environmental Protection Agency Office of Water. Available at: https://www.epa.gov/sites/default/files/2015-10/documents/method_1699_2007.pdf [Accessed 11th May, 2024].

Foley, J.R., IV, Jubb, C.S., Cole, D.A., Mausel, D., Galloway, A.L., Brooks, R. et al. (2021) Historic assessment and analysis of the mass production of *Laricobius* spp. (Coleoptera: Derodontidae), biological control agents for the hemlock woolly adelgid, at Virginia Tech. *Journal of Insect Science*, 21(1), 12.

Foley, J.R., IV, McAvoy, T.J., Dorman, S., Bekelja, K., Kring, T.J. & Salom, S.M. (2019) Establishment and distribution of *Laricobius spp*. (Coleoptera: Derodontidae), a predator of hemlock woolly adelgid, within the urban environment in two localities in southwest Virginia. *Journal of Integrated Pest Management*, 10(1), 30.

Foley, J.R., IV, McAvoy, T.J., Grubb, C., Mayfield, A.E., III, Strahm, B. & Salom, S.M. (2022) Subterranean survivorship and seasonal emergence of *Laricobius* spp. (Coleoptera: Derodontidae), biological control agents for the hemlock woolly adelgid. *Environmental Entomology*, 51(1), 63–70.

Foley, J.R., IV, McAvoy, T.J., Saint-Amant, R., Régnière, J., Biggs, A., Wright, E. et al. (2022) Temperature-dependent development, survival, and oviposition of *Laricobius osakensis* (Coleoptera: Derodontidae): a specialist predator of *Adelges tsugae* (Hemiptera: Adelgidae). *Environmental Entomology*, 51(4), 688–699.

Ford, C.R., Vose, J.M., Daley, M. & Phillips, N. (2007) Use of water by eastern hemlock: implications for systemic insecticide application. *Arboriculture and Urban Forestry*, 33(6), 421–427.

Fossen, M. (2006) *Environmental fate of Imidacloprid*. Environmental Monitoring, Department of Pesticide Regulation. Available at: http://cues.cfans.umn.edu/old/pollinators/pdf-pesticides/2006CAImidclprdfate.pdf [Accessed 20th March 2023].

Gervais, J.A., Luukinen, B., Buhl, K. & Stone, D. (2010) *Imidacloprid general fact sheet*. National Pesticide Information Center, Oregon State University Extension Services. Available at: http://npic.orst.edu/factsheets/imidagen.html [Accessed 20th March 2023].

Gouger, R.J. (1971) Control of *Adelges tsugae* on hemlock in Pennsylvania. *Science Tree Topics*, 3, 1–9.

Grünewald, B. & Siefert, P. (2019) Acetylcholine and its receptors in honeybees: involvement in development and impairments by neonicotinoids. *Insects*, 10(12), 420.

Havill, N.P., Montgomery, M.E., Yu, G., Shiyake, S. & Caccone, A. (2006) Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction to eastern North America. *Annals of the Entomological Society of America*, 99(2), 195–203.

Hillen, A.P., Foley, J.R., IV, Salcedo, M.K., Socha, J.J. & Salom, S.M. (2023) 3D X-ray analysis of the subterranean burrowing depth and pupal chamber size of *Laricobius* (Coleoptera: Derodontidae), a specialist predator of *Adelges tsugae* (Hemiptera: Adelgidae). *Journal of Insect Science*, 23(3), 19.

Kantola, T., Tracy, J., Lyytikäinen-Saarenmaa, P., Saarenmaa, H.T., Coulson, R., Trabucco, A. et al. (2019) Hemlock woolly adelgid niche models from the invasive eastern North American range with projections to native ranges and future climates. *iForest*, 12, 149–159.

Knoepp, J.D., Vose, J.M., Michael, J.L. & Reynolds, B.C. (2012) Imidacloprid movement in soils and impacts on soil microarthropods in southern Appalachian eastern hemlock stands. *Journal of Environmental Quality*, 41(2), 469–478.

Lamb, A.B., Salom, S.M. & Kok, L.T. (2007) Factors influencing aestivation in *Laricobius nigrinus* (Coleoptera: Derodontidae), a predator of *Adelges tsugae* (Hemiptera: Adelgidae). *The Canadian Entomologist*, 139(4), 576–586.

Mayfield, A.E., III, Salom, S.M., Sumpter, K., McAvoy, T., Schneeberger, N.F. & Rhea, R. (2020) *Integrating chemical and biological control of the hemlock woolly adelgid: a resource manager's guide* FHAAST-2018-04 (ed.). Morgantown: USDA Forest Service, Forest Health Assessment and Applied Sciences Team, pp. 1–40.

McAvoy, T., Jubb, C., St-Amani, R. & Salom, S. (2020) *Recovery protocols for Laricobius spp. and Sasajiscymnus tsugae*. Blacksburg: Department of Entomology, Virginia Tech. Available at: https://hwa-pdb-gmsts.hub.arcgis.com/documents/bb0205c020114b95a7350a785569c572/about. [Accessed 3rd February 2021].

McAvoy, T.J., Mays, R., Johnson, N.G. & Salom, S.M. (2017) The effects of shade, fertilizer, and pruning on eastern hemlock trees and hemlock woolly adelgid. *Forests*, 8(5), 156.

McClure, M.S. (1987) *Biology and control of hemlock woolly adelgid*. New Haven, CT: *Connecticut Agricultural Experiment Station*. Available at: https://portal.ct.gov/-/media/CAES/DOCUMENTS/Publications/Bulletins/B851pdf.pdf [Accessed 18th March 2023].

McClure, M.S. (1991) Density-dependent feedback and population cycles in *Adelges tsugae* (Homoptera: Adelgidae) on *Tsuga canadensis*. *Environmental Entomology*, 20(1), 258–264.

Mullins, J.W. (1993) Imidacloprid: a new nitroguanidine insecticide. In: Duke, S.O., Menn, J.J. & Plimmer, J.R. (Eds.) *Pest control with enhanced environmental safety*. Kansas City: American Chemical Society, pp. 183–193.

Nauen, R. & Bretschneider, T. (2002) New modes of action of insecticides. *Pesticide Outlook*, 13(6), 241–245.

Nauen, R., Tietjen, K., Wagner, K. & Elbert, A. (1998) Efficacy of plant metabolites of imidacloprid against *Myzus persicae* and *Aphis gossypii* (Homoptera: Aphididae). *Pesticide Science*, 52(1), 53–57.

Oi, M. (1999) Time-dependent sorption of imidacloprid in two different soils. *Journal of Agricultural and Food Chemistry*, 47(1), 327–332.

Orwig, D.A., Foster, D.R. & Mausel, D.L. (2002) Landscape patterns of hemlock decline in New England due to the introduced hemlock woolly adelgid. *Journal of Biogeography*, 29(10), 1475–1487.

R Core Team. (2021) *R: a language and environment for statistical computing*. Vienna, Austria: *R Foundation for Statistical Computing*. Available at: https://www.R-project.org/ [Accessed 13th February 2023].

Soil Survey Staff. (2023) *Web soil survey*. Natural Resources Conservation Service, United States Department of Agriculture. Available at: http://websoilsurvey.sc.egov.usda.gov/. [Accessed 13th February 2023].

Sparks, T.C., Storer, N., Porter, A., Slater, R. & Nauen, R. (2021) Insecticide resistance management and industry: the origins and evolution of the insecticide resistance action committee (IRAC) and the mode of action classification scheme. *Pest Management Science*, 77(6), 2609–2619.

Steward, V.B., Braness, G. & Gill, S. (1998) Ornamental pest management using imidacloprid applied with the Kioritz [R] soil injector. *Journal of Arboriculture*, 24(6), 344–346.

Sur, R. & Stork, A. (2003) Uptake, translocation, and metabolism of imidacloprid in plants. *Bulletin of Insectology*, 56(1), 35–40.

Vose, J.M., Wear, D.N., Mayfield, A.E., III & Nelson, C.D. (2013) Hemlock woolly adelgid in the southern Appalachians: control strategies, ecological impacts, and potential management responses. *Forest Ecology and Management*, 291, 209–219.

Wamhoff, H. & Schneider, V. (1999) Photodegradation of imidacloprid. *Journal of Agriculture and Food Chemicstry*, 47(4), 1730–1734.

Young, R.F., Shields, K.S. & Berlyn, G.P. (1995) Hemlock woolly adelgid (Homoptera: Adelgidae): stylet bundle insertion and feeding sites. *Annals of the Entomological Society of America*, 88(6), 827–835.

Zilahi-Balogh, G.M.G., Humble, L.M., Lamb, A.B., Salom, S.M. & Kok, L.T. (2003) Seasonal abundance and synchrony between *Laricobius nigrinus* (Coleoptera: Derodontidae) and its prey, the hemlock woolly adelgid (Hemiptera: Adelgidae). *The Canadian Entomologist*, 135(1), 103–115.

Zilahi-Balogh, G.M.G., Salom, S.M. & Kok, L.T. (2003) Development and reproductive biology of *Laricobius nigrinus*, a potential biological control agent of *Adelges tsugae*. *BioControl*, 48, 293–306.

How to cite this article: Hillen, A.P., Foley, J.R. IV, Gross, A.D., Mayfield, A.E. III, Williams, J., Xia, K. et al. (2024) The impact of imidacloprid on the subterranean survivorship of *Laricobius* (Coleoptera: Derodontidae), a biological control agent of *Adelges tsugae* (Hemiptera: Adelgidae). *Agricultural and Forest Entomology*, 1–11. Available from: https://doi.org/10.1111/afe.12647