HEMLOCK WOOLLY ADELGID PHENOLOGY, WINTER MORTALITY, AND SYSTEMIC INSECTICIDE EFFICACY IN *TSUGA CANADENSIS* AND PERSISTENCE IN *AILANTHUS ALTISSIMA*

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ABSTRACT

This thesis, presented in four chapters, focused on quantifying hemlock woolly adelgid (*Adelges tsugae* Annand) (HWA) mortality due to cold winters and insecticide treatments, seasonal phenology, and the persistence of systemic insecticides in eastern hemlock (*Tsuga canadensis* (L.) Carr.), the primary host of HWA, as well as in tree of heaven (*Ailanthus altissima* (Mill.) Swingle), the primary host of the invasive spotted lanternfly (*Lycorma delicatula* White) (SLF). In chapter one, phenology and winter mortality of HWA was observed at sites in Holland and Norton Shores, MI over two winters. Cumulative growing degree days (base 10 °C) associated with HWA life stages were monitored and general risk periods for management activities were established. Winter mortality was observed at both sites in January 2018 and 2019 when temperatures dropped below -20 °C. Data from detection surveys were used to map HWA distribution between 2016 and 2022. In chapter two, mortality of HWA due to systemic imidacloprid or dinotefuran applications was monitored over two years. Dinotefuran treatments provided nearly complete HWA control five months after application. Most imidacloprid applications provided HWA control the following year. Canopy condition of treated and untreated control trees was monitored from 2018 through 2021. In chapter three, persistence of imidacloprid and dinotefuran was quantified in foliage and twig samples from trees treated in 2016/2017 or 2018/2019 using two residue analysis methods. Effects of season and application method on concentrations over time were quantified. Most imidacloprid treatments persisted through 2020 at levels effective for HWA control, while dinotefuran was barely detectable in hemlock tissue. In chapter four, persistence and distribution of dinotefuran in foliage and phloem of tree of heaven were quantified in trees treated in 2019 at two sites. Dinotefuran was not detected in branch phloem and concentrations were inconsistent in trunk phloem.

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CHAPTER 1: WINTER MORTALITY AND DEVELOPMENT OF HEMLOCK WOOLLY ADELGID IN RECENTLY INVADED AREAS OF MICHIGAN

Introduction

Hemlock woolly adelgid (HWA) (*Adelges tsugae* (Annand) (Hemiptera: Adelgidae)), an invasive sap-feeding insect from Japan, was first detected in the eastern United States in Virginia in 1951 (Stoetzel 2002). Populations of HWA are established in 20 eastern states and the Canadian provinces of Ontario and Nova Scotia, extending across approximately half the native range of eastern hemlock (*Tsuga canadensis* (L.) Carriere), as well as the entire range of Carolina hemlock (*T. caroliniana* Engelm.) in North America (CFIA 2023, USDA Forest Service 2023). A distinct lineage of HWA exists in the western United States, which is native to the region and does not cause widespread damage to western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) due to host resistance and the presence of natural enemies (Havill et al. 2016, Jubb et al. 2020).

To feed, adelgids insert their long stylet into the base of hemlock needles to suck sap and nutrients from xylem ray parenchyma cells lining the vascular tissue in shoots (Young et al. 1995). Adelgids secrete white waxy filaments as they feed, which provide protection from desiccation and give the insects their “woolly” appearance (McClure 1989, McCullough et al. 2015). Feeding by HWA can cause needles to desiccate and drop, and buds may die on infested shoots. Tree mortality can occur within 2 to 12 years of initial infestation, depending on HWA density, tree size and tree condition (McClure et al. 2001, Ward et al. 2004). In the U.S., HWA completes two parthenogenic generations annually. Progrediens, which hatch in late spring, feed and mature by mid-summer. Eggs laid by progrediens hatch within a few days into sistens crawlers (first instars), which settle on branches or twigs, then aestivate during the summer as nymphs. Sisten nymphs begin feeding in fall and continue to feed and develop through winter (Havill et al. 2016, McClure 1989, McClure et al. 2001).

Eastern hemlock is considered an ecological foundation species and is often a major overstory component in northern hardwood stands and riparian forests (Ellison et al. 2005a, Orwig et al. 2012). Hemlocks exert substantial effects on soil pH and nitrogen cycling (Ellison et al. 2005a, Jenkins et al. 1999, Martin and Goebel 2013, Yorks et al. 2000) and are especially important in riparian areas, where they reduce water temperature, riverbank erosion and nutrient leaching (Ellison et al. 2005a). Many wildlife species rely on dense hemlock canopies for habitat, cover, or food; whitetailed deer preferentially browse hemlock shoots and regeneration (Ellison et al. 2005a, Frelich and Lorimer 1985, Ward et al. 2004,). In Michigan, which is on the western edge of the eastern hemlock range, hemlocks are notably important for stabilizing dunes along Lake Michigan (West Michigan HWA Taskforce 2022).

Hemlock woolly adelgid was reported in Michigan on private property in Ottawa and Muskegon Counties in 2015 (MDARD 2016). Discussions with property owners in Ottawa County indicated nursery trees imported from eastern states in the 1990s may have introduced HWA in the early or mid 1990’s, but there are no records to confirm this (McCullough et al. 2019). Ongoing detection surveys subsequently identified additional localized infestations in six counties, all within approximately 25 km of the Lake Michigan shoreline (MDNR 2023, Figure 1.6). Michigan forests have more than 173 million hemlocks threatened by HWA (USDA Forest Service 2020). A multi-agency effort to detect and contain HWA infestations was launched in Michigan in 2017 with the intention of containing HWA and slowing its spread into the northern Lower and Upper Peninsulas where most of the hemlock is found (Kiefer et al. 2022).

Current HWA distribution may reflect the influence of Lake Michigan on regional climatic variables, particularly winter temperatures experienced by the sistens generation (Kiefer et al. 2022). When cold surface air crosses the 58,000 ha Lake Michigan, energy is released producing cloudy, insulating conditions that moderate air temperatures along the lakeshore and in downwind areas (Albert et al. 1986, Andresen et al. 2012). Because of this Lake Effect weather, minimum winter temperatures within 48 km of the lakeshore can be up to 6 °C warmer than inland areas of Michigan at the same latitude (Andresen et al. 2012).

Winter mortality of sistens has been reported in previous studies (Havill et al. 2016, McClure 1989, McClure et al. 2001, Tobin et al. 2017), which suggests the influence of Lake Michigan may contribute to survival of HWA sistens, at least in some years. In laboratory studies, HWA sustained up to 94 % mortality when exposed to -20 °C, and 100 % mortality when exposed to -35 °C and lower (Gouli et al. 2000, Parker et al. 1998, Parker et al. 1999). Field studies similarly identified -20 °C as a temperature threshold associated with severe or nearly complete HWA mortality (Costa et al. 2008), potentially limiting HWA range expansion in northern latitudes or at high elevations (McAvoy et al. 2017, Paradis et al. 2007, Tobin et al. 2017, Trotter and Shields 2009). Gradual exposure to increasingly cold temperatures, however, may initiate the production of cryoprotectants in HWA hemolymph that protect against freezing (Elkinton et al. 2017). Sharp temperature drops over short periods of time may, therefore, also play a role in HWA winter mortality if HWA sistens are not cold-acclimated (McAvoy et al. 2017). There is also evidence indicating that HWA populations in the northeastern US may be adapting on a genetic level to survive cold winter temperatures (Butin et al. 2005). Rates of winter survival of HWA in Michigan and the potential role of Lake Effect weather, however, have not been previously assessed.

The probable establishment of HWA in Michigan approximately 20 years before populations were detected, and the current HWA distribution, still largely limited to sites within 25 km of Lake Michigan, has provoked considerable interest. Managers currently dealing with HWA infestations in Michigan are obviously interested in estimates of the extent and frequency of HWA winter mortality, which could reduce density and perhaps slow expansion of existing HWA infestations or spread into new areas. In addition, forest managers have requested information about the onset and duration of HWA life stages in Michigan for planning insecticide applications, scheduling detection or delimitation surveys or avoiding periods when HWA eggs or crawlers could be accidentally introduced into new areas.

Previous studies to monitor HWA development in the eastern U.S. reported calendar dates associated with HWA life stages (Gray and Salom 1996, Havill et al. 2016, McClure 1989, McClure et al. 2001). However, calendar dates fail to account for regional or annual differences in temperatures, making it difficult to apply observations to different regions. Degree days (DD) are calculated using a base threshold temperature, above which insect development begins to occur. Base temperatures can vary by species and among populations, but most are reasonably approximated with a base 10 °C (50 °F) or in some cases, a base 0 °C threshold temperature (Herms 2004). In a laboratory study, Salom et al. (2002) identified 4 °C as a base temperature threshold for the onset of HWA development. When they monitored HWA phenology at two field sites in VA for a year, cumulative DDs required for progrediens development from nymph to adult, however, was 64 to 68 % of DD accumulation required in the laboratory setting. The extent of variation in DD accumulation or HWA development between the two sites was not reported. Tobin and Turcotte (2018) used the 4 °C temperature threshold to model HWA development in four sites in the Appalachian Mountains across two years. Observations were pooled, however, and it was unclear if DD accumulation varied among sites or between years. Additionally, few publicly accessible sources of weather data report cumulative DD using 4 °C as a base temperature, limiting application of results from these studies.

In contrast, cumulative DDs generated with a base temperature of 10 °C are widely available and commonly used in agriculture and natural resources for pest scouting or management activities. Real time and projected degree day accumulations using a base threshold of 10 °C are provided on numerous publicly accessible websites such as those maintained by land grant universities and agencies that track weather (e.g., MSU EnviroWeather, MSU MAWN, National Weather Service [NOAA 2023], Purdue Agricultural Data Engine 2023, USA National Phenology Network 2022). The ability to anticipate HWA life stages and delineate high-risk periods by monitoring cumulative growing degree days would be useful for managers, most of whom lack the ability to monitor temperatures and degree day accumulation on their own, particularly across multiple locations.

Our objectives in this study included identifying periods when HWA feeding and reproduction occurred and evaluating effects of extreme minimum temperatures on sistens survival in western Michigan. We monitored HWA life stages on hemlocks in two forested sites, both located < 10 km from the Lake Michigan shoreline and identified cumulative degree days (base 10 °C) corresponding to our observations. We also present annual detections of localized HWA infestations between 2016 and 2022 and estimate linear distances between new and previously identified HWA populations.

Materials and Methods

Study Sites: We conducted this study in two sites where HWA was detected in 2016. The 16 ha Norton site consisted of a central, relatively flat campground with semi-permanent trailers surrounded by steep, forested dunes near Norton Shores, Muskegon County, MI, less than 0.5 km east of Lake Michigan. Overstory composition was dominated by mature eastern hemlock, eastern white pine (*Pinus strobus* (L.)), American beech (*Fagus grandifolia* (Ehrh.)), northern red oak (*Quercus rubra* (L.)), and sugar maple (*Acer saccharum* (Marshall)). Understory composition was sparse and comprised primarily of tree seedlings and saplings.

The 4 ha Holland site in Holland, Ottawa County, MI, was in a forested area in a large county park, approximately 48 km south of the Norton site and 6.5 km east of Lake Michigan. Overstory composition was similar to Norton, but a dense understory of beech saplings and raspberry (*Rubus strigosus* (Michx.)) covered much of the site.

Development and Mortality of HWA: To monitor HWA life stages and assess HWA mortality, a single infested, foliage-bearing shoot, 10 to 30 cm long, was collected from the lower canopy of ten trees at the Norton site at approximate four-week intervals from 2 November 2016 to 10 August 2017. Shoots were individually bagged, labeled, and returned to the MSU Forest Entomology Laboratory in sealed containers, in compliance with protocols approved by the Michigan Department of Agriculture and Rural Development. After shoot length was measured, individual shoots were examined under a microscope. We counted adelgid ovisacs, eggs, first-instar crawlers, and settled nymphs then standardized counts per linear meter. Density and proportion of HWA by life stage were calculated for each sampling period and site. Due to our unfamiliarity with HWA life stages at that time and the four-week sampling intervals, these observations were treated as preliminary data and were not further analyzed.

More intensive sampling to monitor HWA density and development was conducted from 31 October 2017 to 26 July 2018. A total of 12 infested, foliage-bearing shoots, each 10 to 30 cm long, were collected from each of two trees at two-week intervals at both the Norton and Holland sites. When possible, shoots were collected from four cardinal aspects and three heights (< 1m, ~2 m, ≥ 3m). Shoots from each tree were individually bagged, returned to the MSU Forest Entomology Laboratory, measured and examined as above. Ovisacs from previous seasons, distinguished by the dark, chalky texture of wax and either a heavily desiccated adelgid or no adelgid, were also recorded. Number and condition of HWA life stages were recorded again from 16 November 2018 to 27 August 2019 using the same methods as in the previous year.

Cumulative DDs corresponding to our first observations of HWA life stages were acquired each year from an on-site weather station at the Norton site (see below) and from the MSU Enviroweather station at West Olive, MI, approximately 21 km northeast of the Holland site. Degree days were calculated with the Baskerville-Emin method with a starting date of 1 January for each year and a base temperature of 10 °C.

Winter Temperatures and HWA Mortality: A weather station deployed at the Norton site (CR1000, Campbell Scientific Inc., Logan, UT) tracked temperatures from 20 October 2017 to 3 March 2021. The weather station deployed at the Holland site (HOBO RX3000, Onset Computer Corporation, Bourne, MA) recorded temperatures from 27 September 2017 to 15 May 2018, 25 October 2018 to 14 May 2019, and 25 October 2019 to 18 June 2020. The property manager required that this weather station be retrieved annually in spring, and it was re-installed at the same location each fall. Stations at both sites were set up in an open area within 3 m of a hemlock tree with live branches on all four cardinal aspects and one or more live, foliage-bearing branches within 0.5 m of the ground. An ambient temperature sensor with a solar shield was installed on top of each station. Stations were powered by solar panels and recorded temperatures at 5-minute intervals. Temperature data were downloaded weekly.

We continued to collect shoots from infested hemlock trees at 2 to 3 week intervals (depending on site accessibility) during the winter in 2017-2018 and 2018-2019 to record number of live and dead HWA sistens. Condition of sistens on the shoots was determined by positioning the shoot under a microscope, gently removing wax, then puncturing each adelgid with a pin. Adelgids that were easily punctured or produced bright hemolymph were tallied as alive, while dead adelgids resisted puncturing, had a spongy texture, or produced dark, chunky hemolymph. Mean, minimum, and maximum ambient temperatures recorded from 21 December to 20 March (winter to spring solstice) in 2017 to 2018, 2018 to 2019, and 2019 to 2020, along with duration of time with temps $\leq$ -20 °C, were related to counts of live and dead sistens.

Detections of HWA 2016-2022: The Michigan Department of Natural Resources (MDNR) provided GPS coordinates of positive HWA detections from field surveys conducted by personnel from the MDNR and West Michigan Cooperative Invasive Species Management Area from 2016 to 2022. Annual HWA distribution maps were created with ArcGIS Pro and distances between the new and the closest previous-year detections were measured.

Data Analysis: Counts of live sistens and progrediens from fall 2017 to 2019 were summed and standardized per meter for both the Norton and Holland sites. Sistens were defined as actively feeding HWA observed from the first sample date in autumn until 31 May of the following year, while progrediens were defined as adelgids active between 1 June to the last sample date in late July or August. Population growth rates between 2017 and 2019 at Norton and Holland were estimated by dividing the density of each generation, standardized by the number of live adelgids observed per meter of shoot, by that of the previous generation, following methods of Tobin et al. (2017).

Winter mortality of HWA sistens was analyzed separately for the 2017 to 2018 and 2018 to 2019 winters. A generalized linear mixed model with a binomial distribution (PROC GLIMMIX, SAS 9.4) was conducted to assess effects of site, date, and their interaction on the proportion of dead HWA on shoots collected during the winter. When effects were significant, Tukey's multiple comparison test was used to separate means among dates.

Results

Development and Mortality of HWA: Sistens that aestivated on hemlock shoots during the summer moved to the base of needles and were actively feeding when we first sampled shoots at the Norton site on 31 October in 2017 and again on 31 October 2018, corresponding to approximately 1600-1650 cumulative degree days (DDs) (base 10 ºC) (Table 1.1). At the Norton site, average densities of live sistens during the 2017-2018 and 2018-2019 winters were similar (Table 1.2). The highest number of sistens observed on a single shoot at the Norton site occurred in November during the 2017-2018 winter and in January during the 2018-2019 winter. Average densities of sistens on the shoots collected on those dates were approximately 6 and 16 times greater, respectively, than the average densities recorded across shoots examined throughout the two winters (Table 1.2). Eggs laid by sistens were first observed at the Norton site between mid-April and early May each year, corresponding to 1 to 73 cumulative $DD_{10℃}$ (Table 1.1). Average density of eggs laid by sistens varied considerably among shoots (Table 1.2), especially as an increasing proportion of adelgids matured. Highest egg densities recorded on individual shoots occurred on dates corresponding to approximately 58 and 165 $DD_{10℃}$ in 2018 and 2019, respectively. These densities were 24 and 7.5 times higher than egg density averaged across all shoots examined in 2018 and 2019, respectively (Table 1.2). On average, individual ovisacs contained 21 ± 4 eggs in 2018 and 28 ± 4 eggs in 2019.

Feeding and development of sistens at the Holland site were similar in timing to sistens at the Norton site. As at Norton, sistens were actively feeding at the first fall sampling on 31 October in both 2017 and 2018, corresponding to 1600-1650 DD$_{10C}$ (Table 1.1). Average density of live sistens recorded on shoots collected from trees at Holland was four times higher during the 2017-2018 winter than during the 2018-2019 winter (Table 1.2). Peak densities of sistens were recorded on shoots collected in early February 2018 and in mid January 2019, when counts were seven and 12 times higher, respectively, than sistens densities averaged across all shoots examined the respective winter periods (Table 1.2). Sistens began laying eggs by mid to late April in both 2018 and 2019, much like the Norton site. Egg density varied substantially among shoots collected in spring, but peaked in mid and late May in 2018 and 2019, respectively (Table 1.2). Maximum egg densities were six to seven times higher than the season-long average in 2018 and 2019 (Table 1.2). Number of eggs per ovisac averaged 51 ± 10 eggs in 2018 but only 17 ± 3 eggs in 2019.

At the Norton site, much of the feeding and development of progrediens occurred in May and June in both 2018 and 2019 (Table 1.1) and there was considerable overlap among life stages (Fig. 1.1). We observed progrediens crawlers moving about on shoots collected at the Norton site in mid to late May in both 2018 and 2019, corresponding to approximately 58 to 104 cumulative DD$_{10°C}$ (Table 1.1). Average crawler densities were nearly identical on shoots collected in spring 2018 and 2019, but maximum counts of crawlers were almost three times higher in 2018 than in 2019 (Table 1.2). Our first observations of settled, feeding progrediens nymphs at Norton occurred between late May and mid-June, corresponding to 165 to 180 cumulative DD$_{10°C}$ (Table 1.1). As with crawler counts, densities of settled nymphs were substantially higher in spring 2018 than in 2019 (Table 1.2). Counts of nymphs in spring were challenging given the ability of crawlers to drop off shoots or perhaps hide under ovisacs when disturbed while examining shoots under a microscope. Adult progrediens with eggs were not observed on shoots until June in both 2018 and 2019. Densities of adult progrediens were much lower than counts of nymphs in May in 2018 and 2019, suggesting many immature adelgids died (Table 1.2). We observed eggs in ovisacs of mature progrediens in late June, corresponding to 251-416 cumulative $DD_{10°C}$ in 2018 and 2019 (Table 1.1). The number of eggs per progrediens ovisac averaged 6 ± 1 in 2018 and 19 ± 3 in 2019.

At the Holland site, observations and densities of progrediens were similar to those at the Norton site and life stages often overlapped (Table 1.2, Fig. 1.2). Average crawler densities varied considerably among shoots and sampling dates and, as at Norton, counts of nymphs were higher than crawler counts. Adult densities were generally higher at Holland than at Norton (Table 1.2) and eggs were observed later than they were at the Norton site (Table 1.1). Average egg counts per progrediens ovisac were relatively consistent at the Holland site among shoots in both years. Number of eggs per progrediens ovisac averaged 4 ± 0.32 and 3 ± 0.48 eggs per ovisac in 2018 and 2019, respectively.

Sistens crawlers began hatching from eggs laid by progrediens between late June and mid to late July at both the Norton and Holland sites. Cumulative degree days associated with our first observations of sistens crawlers were consistent within each site, at around 600 $DD_{10°C}$ at the Norton site each year, and 700 $DD_{10°C}$ at the Holland site (Table 1.1). Our counts of sistens crawlers on shoots collected during the summer were consistently low (Table 1.2), but settled, feeding nymphs were easier to find. Average densities of sistens nymphs were relatively consistent at both sites in both 2018 and 2019 (Table 1.2).

Minimum Winter Temperatures and HWA Sistens Mortality - 2017-2018: Winter temperatures from 21 Dec 2017 to 20 March 2018 at the Norton site ranged from -19 to 10 °C and averaged -4.0 °C ± 0.03. The average minimum temperature was -6.7 °C ± 0.5, average maximum temperature was -0.4 °C ± 0.6, and average daily temperature was -3.7 °C ± 0.5, respectively. December had the lowest average monthly temperature, but the minimum temperature for the winter occurred on 5 January 2018 (Table 1.3). Temperatures dropped 9 °C in the 24-hour period before this minimum temperature of -19 °C was recorded. Temperatures of -18 to -19 °C persisted for an hour.

Mortality of sistens on shoots collected from trees at the Norton site between 21 Dec 2017 and 20 March 2018 ranged from 0 to 30 %, with an average of 13.0 ± 2.00 % (Figure 1.3). We did not observe high HWA mortality at any point during the sistens feeding period and winter temperature never dropped below -20 °C. Over the winter, 5 % of the shoots we examined had only dead or old HWA ovisacs; 95% of the shoots had one or more live adelgid.

Temperatures at the Holland site from 21 Dec 2017 to 20 March 2018 ranged from -21 to 17 °C and averaged -2.0 °C ± 0.04. Average minimum temperature was -5.9 °C ± 0.6, average maximum 1.6 °C ± 0.7, and average daily temperature was -2.2 °C ± 0.6. December had both the lowest average temperature, as well as the lowest minimum temperature of the winter (Table 1.3). Temperatures dropped 14 °C over a 24 hour span from 30 Dec to 31 Dec 2017. The minimum temperature of -21 °C was recorded on 31 Dec 2017 and persisted for two hours. This temperature was two degrees colder than the lowest minimum temperature recorded at the Norton site on any date.

Significant mortality of sistens on shoots from trees at the Holland site was observed on shoots collected between 21 December 2017 and 20 March 2018. On 22 February 2018, 45.0 ±

8.00 % sistens mortality was recorded, significantly higher than the mortality observed at the Norton site on the same day ($P < 0.0001$). On 6 March 2018, an average of 95.0 ± 2.00 % of sistens on shoots we examined were dead and mortality remained high on shoots collected on 20 March, ranging from 82 to 100 % (Figure 1.3). Our estimated HWA population growth rate calculations showed the sistens population density dropped compared to the preceding progrediens generation (Figure 1.5). Over the entire winter, 12 % of the shoots we examined only had dead or old ovisacs present on them.

Minimum Winter Temperatures and HWA Sistens Mortality - 2018-2019: From 21 Dec 2018 to 20 March 2019, winter temperatures at the Norton site ranged from -24 to 12 °C and averaged -4 °C ± 0.03. average minimum temperature was -7.5 °C ± 0.60, while average maximum temperature was -1 °C ± 0.50, and average daily temperature was -4.2 °C ± 0.50. January was the coldest month during the winter at the Norton site, with the lowest extreme minimum and lowest average temperatures (Table 1.3). Temperatures dropped below -20 °C on two dates. On 21 January 2019, temperatures dropped 14 °C within 24-hours, reaching a low of -24 °C. Temperatures remained below -20 °C for nearly nine hours (Figure 1.4). On 30 January 2019, temperatures dropped 11 °C in a 24-hour period to a minimum temperature of -21 °C and remained below -20 °C for five hours.

Mortality of sistens was monitored at the Norton site between 21 December 2018 and 20 March 2019. Over the winter, 64 % of shoots collected from trees at Norton had only dead or old sistens ovisacs, higher than the previous year. Mortality of HWA on shoots collected on 6 February 2019 averaged 96.0 ± 2.00 % (Figure 1.4) and ranged from 74 to 100 %, significantly higher than the mortality observed on 17 January 2019 ($P < 0.0001$). Estimated population density of sistens was high compared to both the preceding progrediens generation, and the progrediens generation that followed (Figure 1.5).

At the Holland site, temperatures from 21 Dec 2018 to 20 March 2019 ranged from -23 to 17 °C and averaged -3.0 °C ± 0.04. average minimum temperature was -6.3 °C ± 0.60, average maximum temperature 0.9 °C ± 0.60, and average daily temperature -2.8 °C ± 0.60. The lowest minimum and average temperatures during the 2018-2019 winter both occurred in January (Table 1.3). On 21 January 2019, the minimum temperature reached -23 °C and stayed below -20 °C for four hours. The temperature dropped 14 °C over a 24-hour period.

Mortality of sistens at the Holland site was monitored between 21 December 2018 and 20 March 2019. From 6 February to 12 March 2019, sistens mortality on shoots we collected averaged 61.0 ± 9.00 and 93.0 ± 3.00 %, respectively (Figure 1.4). Mortality of HWA on individual shoots during this period ranged from 50 to 100 % on 6 February and 67 to 100 % on 12 March 2019. Estimated population growth rate of the sistens generation at this time was in decline, and this trend continued to the following progrediens generation at the Holland site (Figure 1.5).

HWA Detections 2015-2022: The first established HWA infestation was detected on private land in 2015 in Ottawa Co., in southwest lower Michigan. Additional infestations were identified in Ottawa County the following year, all within 5.5 km from the initial detection (Figure 1.6). A multi-agency HWA program involving the Michigan Department of Natural Resources, the Michigan Department of Agriculture and Rural Development, the West Michigan CISMA and other groups was launched in 2017. This effort encompassed detection and delineation surveys, systemic insecticide applications and public outreach. Additional localized infestations continued to be identified in Ottawa County in 2017 but much of the focus shifted north in 2018 and 2019, as populations were discovered in Muskegon and Oceana Counties (Figure 1.6). In 2020, HWA infestations were identified in a state park in Mason Co., 23 km north of the nearest detection in Oceana Co. (Figure 1.6). Infestations continued to be identified from 2021 to 2022, reflecting the ongoing progress of the surveys to the north and outreach activities targeting landowners, arborists and personnel at parks and recreation areas. The linear distance from the original detection points in 2015 to the furthest northern point in 2022 was approximately 84 km, averaging 12 km of northern spread per year along the Lake Michigan shoreline. However, HWA infestations were never detected more than 15 km east of the Lake Michigan shoreline (Figure 1.6).

Discussion

The phenology of HWA in Michigan was relatively consistent in both sites monitored over two years in southwest Michigan. Sistens were feeding by late October, corresponding to approximately 1600-1650 DD_{10C} and they continued to feed and develop during the winter until oviposition began in mid- to late-April, as soon as degree days began to accumulate (1-44 DD_{10C}). Crawlers, the mobile first stage nymphs, were active throughout May (58-161 DD_{10C}). Given the abundance of crawlers we observed in early to mid-May, egg hatch likely began by late April, between observation dates. Progrediens fed in May and June (165-279 DD_{10C}) and matured by late June or early July, when eggs were observed in ovisacs (463-543 DD_{10C}). There was some overlap in spring and early summer in eggs, newly hatched progrediens crawlers, and settled nymphs, reflecting in part our 2-week sampling intervals, as well as microclimatic variation in temperatures experienced by adelgids (Kiefer et al., in prep.). Although we cannot conclusively identify the onset of different life stages, our data provide information relevant for operational management activities. For example, surveys will most likely detect new, low density

HWA infestations when ovisac wax is most abundant just prior to, and during, oviposition. This would be approximately 1-50 DD_{10C} in the early spring, and again from 300-600 DD_{10C} in the summer when progrediens do the same. Personnel should be especially cautious when working in infested areas during periods when crawlers are abundant, at approximately 60-160 DD_{10C} for progrediens crawlers, and again at 600-750 DD_{10C} for sistens crawlers to prevent unintended dispersal.

Development of HWA life stages in Michigan varied from some previous reports or phenological models, reinforcing the need to monitor local populations rather than extrapolating from observations in other regions (Tobin and Turcotte 2018). Phenological forecasts to predict occurrence of life stages of various invasive pests based on data from the USA National Phenology Network, suggested HWA sistens would lay eggs at 25 cumulative DDs. In this case, degree days were calculated using a base threshold temperature of 0 °C, a start date of 1 January and a simple averaging accumulation method (Crimmins et al. 2020). In Georgia, eggs laid by sistens were first observed in February (Joseph et al. 2011), only a few weeks earlier than projections by Crimmins et al. (2020). In the two Michigan sites we monitored, however, 25 cumulative DDs calculated with a 0 °C base temperature would have occurred in January, whereas we did not observe eggs laid by sistens until mid to late April. Our study utilized the publicly available MSU Enviroweather Network, a meso-net consisting of more than 90 weather stations located around the state and associated online tools. Degree days are calculated using the Baskerville-Emin method with a January 1 start date and a 10 °C base temperature threshold. The Baskerville-Emin method of calculating DD accumulation fits a sine curve to maximum and minimum daily temperatures, reflecting temperature warming during the day and cooling at night. In a previous laboratory study, HWA development began at 4 °C (Salom et al. 2002), a threshold subsequently used in the phenological study conducted by Tobin & Turcotte (2018). In our study, high-risk periods such as presence of HWA eggs or crawler activity, were identified and linked with the corresponding accumulation of DDs calculated with the 10 °C base threshold. This threshold facilitates access to DD accumulations from local weather stations, including those maintained by MSU in our case, as well as other publicly available sources. Our results can be applied more broadly to management activities outside of Michigan without requiring on-site weather stations and the need to manually calculate degree day using other base threshold temperatures.

Inland spread of HWA, i.e., to the east of the Lake Michigan shoreline, has been much slower (no more than 15 km east over seven years of surveys) than the reports of HWA estimated in other regions, which ranges from 8.9 to 15.6 km per year (Evans and Gregoire 2007, Fitzpatrick et al. 2010, Morin et al. 2009). Previous studies have suggested that HWA spread varies geographically, with slower spread rates in colder northern U.S. regions and faster in southern regions (Evans and Gregoire 2007, Morin et al. 2009). Northern spread in our study along the Lake Michigan shoreline, at approximately 12 km per year was more in line with the 12.5 km spread estimated by Evans and Gregoire (2007). Given that HWA infestations are currently confined to areas within 15 km of the Lake Michigan shoreline, it seems likely that colder inland temperatures, where winter temperatures below -20 °C are common, limit HWA establishment or spread (Kiefer et al. 2022). High HWA mortality associated with temperatures of -20 °C has been widely reported in laboratory and field studies (Cheah 2017, Costa et al. 2008, Gouli et al. 2000, Parker et al. 1998, Parker et al. 1999). In our studies, unusually high HWA sistens mortality was observed in 2018 at the Holland site, and at both the Holland and Norton sites in 2019. In each of these instances, minimum temperatures dropped to below -20 °C in January. To further illustrate the importance and validity of this threshold temperature, in 2018 when high mortality was observed at the Holland site but not at Norton, the difference between extreme minimum temperatures was 2.2 °C and the -19 °C minimum temperature recorded at Norton was only 1 °C shy of the -20 °C threshold. Mortality rates for sistens at the Norton site were similar before and after the -19 °C temperature was recorded and never exceeded approximately 15 % during the winter. In contrast, sistens mortality at Holland was nearly 100 % following the January night with -21.2 °C temperatures.

Temperatures below -20 °C are much less frequent along Lake Michigan in the Lower Peninsula of Michigan, where our two study sites were located, compared with most of the Upper Peninsula as well as the inland, north central portion of the Lower Peninsula of Michigan (Contosta et al. 2019, Kiefer et al. 2022, McAvoy et al. 2017). A recent climate study in the Great Lakes region showed that between 1981-2018, the area within 30 km of the Lake Michigan shoreline experienced -20 °C temperatures an average of one to seven days per year. As climate change progresses, however, the area defined by a -20 °C isotherm is projected to shift inland by 25-50 km in a best-case and 50-75 km in a worst-case expansion scenario and by late century, cold days will be infrequent or even non-existent (Kiefer et al. 2022). McAvoy et al. (2017) similarly predicted warmer winter temperatures in high latitude regions and suggested that the eastern shore of Lake Michigan, with a current projected HWA winter mortality percentage of 70-80 %, would decrease to 30-50 % by late century. In the span of our study, the winter of 2017-2018 had no days below -20 °C at the Norton site, and one day at the Holland site. In the 2018-2019 winter, two days that reached -20 °C at the Norton site, and one day at the Holland site. However, frequency of extremely cold days varies considerably. During the 2019-2020 winter, minimum temperatures near our study sites did not drop below -20 °C. In the 2020-2021 winter, two consecutive days with temperatures at -20 °C occurred in February 2021. Although HWA mortality was not monitored regularly, shoots examined in March 2021 showed that approximately 50-60% of sistens had died. Previous research, however, suggests that HWA densities can rebound if 2 to 9 % of sistens survive (Paradis et al. 2008, Trotter and Shields 2009), reflecting reproduction of progrediens that are unaffected by cold temperatures. While spread of HWA to the extensive hemlock resources in north central Lower Michigan and much of Upper Michigan may be slow relative to other states, it seems likely that HWA populations will persist near Lake Michigan and benefit from ongoing changes in winter weather driven by climate change.

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Table 1.1. Dates and corresponding degree days (base 10 °C)[1] associated with first observations of sistens and progrediens life stages on shoots collected at two-week intervals in the Norton and Holland sites from 2017 to 2019.

		Sistens		**Progrediens**			**Sistens**	
Norton site		**Feeding**[2]	**Eggs**	**Crawlers**	**Nymphs**[3]	**Eggs**	**Crawlers**	**Nymphs**
2017-2018	**Calendar date**	31 Oct	18 April	15 May	31 May	28 June	12 July	26 July
	DD$_{10° \mathrm{C}}$	1596	1	58	180	416	595	756
2018-2019	**Calendar date**	31 Oct	4 May	31 May	11 June	17 July	31 July	31 July
	DD$_{10° \mathrm{C}}$	1649	32	104	165	463	616	616
Holland site								
2017-2018	**Calendar date**	31 Oct	18 April	15 May	31 May	28 June	12 July	26 July
	DD$_{10° \mathrm{C}}$	1596	9	115	265	538	731	901
2018-2019	**Calendar date**	31 Oct	23 April	31 May	18 June	11 July	24 July	24 July
	DD$_{10° \mathrm{C}}$	1649	44	161	279	543	699	699

[1] Cumulative degree days were acquired from the on-site weather station at the Norton site and the MSU Enviroweather station at West Olive, MI for the Holland site. Degree days were calculated using the Baskerville-Emin method with a base threshold of 10 °C and start date of 1 January each year.

[2] Feeding sistens were characterized by ovisac formation, indicating active feeding and wax filament secretion.

[3] Nymphs were immature adelgids after crawlers had dispersed; characterized by a white "halo" around the adelgid but lacking wax filaments.

Table 1.2. Mean (± SE) and maximum density of HWA life stages (number m^{-1}) on shoots collected at 2-week intervals at the Norton and Holland sites in 2017-2019 (n = 12 shoots per site each sample date).

		Sistens		**Progrediens**				**Sistens**	
Norton site		**Feeding**	**Eggs**	**Crawlers**	**Nymphs**	**Adults**	**Eggs**	**Crawlers**	**Nymphs**
2017-2018	**Mean ± SE**	47 ± 4	284 ± 135	43 ± 18	202 ± 34	11 ± 5	80 ± 28	12 ± 5	81 ± 34
	Max	292	6826	680	1282	167	667	103	423
	Date of Max	28 Nov	15 May	15 May	12 June	28 June	28 June	12 July	26 July
2018-2019	**Mean ± SE**	34 ± 7	446 ± 167	43 ± 16	100 ± 20	8 ± 4	252 ± 120	16 ± 8	71 ± 22
	Max	568	3343	229	237	67	1844	67	169
	Date of Max	17 Jan	11 June	11 June	25 June	25 June	25 June	25 June	17 July
Holland site									
2017-2018	**Mean ± SE**	97 ± 10	549 ± 151	23 ± 8	232 ± 21	41 ± 10	211 ± 42	28 ± 7	360 ± 58
	Max	667	3488	236	524	341	939	140	1067
	Date of Max	8 Feb	15 May	15 May	31 May	28 June	28 June	12 July	26 July
2018-2019	**Mean ± SE**	25 ± 4	360 ± 107	80 ± 25	179 ± 35	17 ± 5	79 ± 30	3 ± 1	356 ± 74
	Max	317	2678	291	551	97	400	17	881
	Date of Max	15 Jan	31 May	31 May	24 July	24 July	24 July	7 Aug	7 Aug

Table 1.3. Mean (±SE), minimum, and maximum ambient temperatures (ºC) recorded at 5-minute intervals by on-site weather stations from 21 December 2017 to 20 March 2018, and from 21 December 2018 to 20 March 2019 at the Norton and Holland Sites. Dates corresponding to minimum temperatures and the drop in temperature recorded during the preceding 24 hours leading to those temperatures are also presented.

	Norton Site		**Holland Site**	
December	**2017 -2018**	**2018 - 2019**	**2017 -2018**	**2018 - 2019**
Mean	-8.6 ± 0.09 ºC	-0.04 ± 0.04 ºC	-7.5 ± 0.09 ºC	1.0 ± 0.05 ºC
Min	-17.4 ºC	-5.2 ºC	-21.2 ºC	-3.3 ºC
Max	1.3 ºC	7.7 ºC	2.1 ºC	12.2 ºC
Date of Min	31-Dec	29-Dec	31-Dec	25-Dec
Temp difference during preceding 24 hrs	8.9 ºC	12.9 ºC	14.1 ºC	4.3 ºC
January				
Mean	-4.1 ± 0.06 ºC	-6.1 ± 0.07 ºC	-2.7 ± 0.07 ºC	-4.8 ± 0.07 ºC
Min	-19.0 ºC	-24.2 ºC	-16.1 ºC	-22.5 ºC
Max	7.3 ºC	6.1 ºC	14.6 ºC	9.3 ºC
Date of Min	5-Jan	21-Jan	4-Jan	21-Jan
Temp difference during preceding 24 hrs	9.3 ºC	14.4 ºC	9.8	15.3
February				
Mean	-3.2 ± 0.06 ºC	-4.7 ± 0.05 ºC	-1.2 ± 0.07 ºC	-3.0 ± 0.06 ºC
Min	-15.3 ºC	-18.3 ºC	-13.3 ºC	-17.9 ºC
Max	10.1 ºC	6.7 ºC	17.3 ºC	10.6 ºC
Date of Min	13-Feb	1-Feb	13-Feb	1-Feb
Temp difference during preceding 24 hrs	11.2 ºC	3.8 ºC	10.7 ºC	4.8 ºC
March				
Mean	-1.1 ± 0.04 ºC	-3.0 ± 0.07 ºC	0.04 ± 0.04 ºC	-1.5 ± 0.07 ºC
Min	-8.3 ºC	-16.2 ºC	-7.7 ºC	-13.5 ºC
Max	8.4 ºC	12.4 ºC	10.6 ºC	16.9 ºC
Date of Min	10-Mar	8-Mar	10-Mar	4-Mar
Temp difference during preceding 24 hrs	9.3 ºC	12.1 ºC	9.6 ºC	10.5 ºC

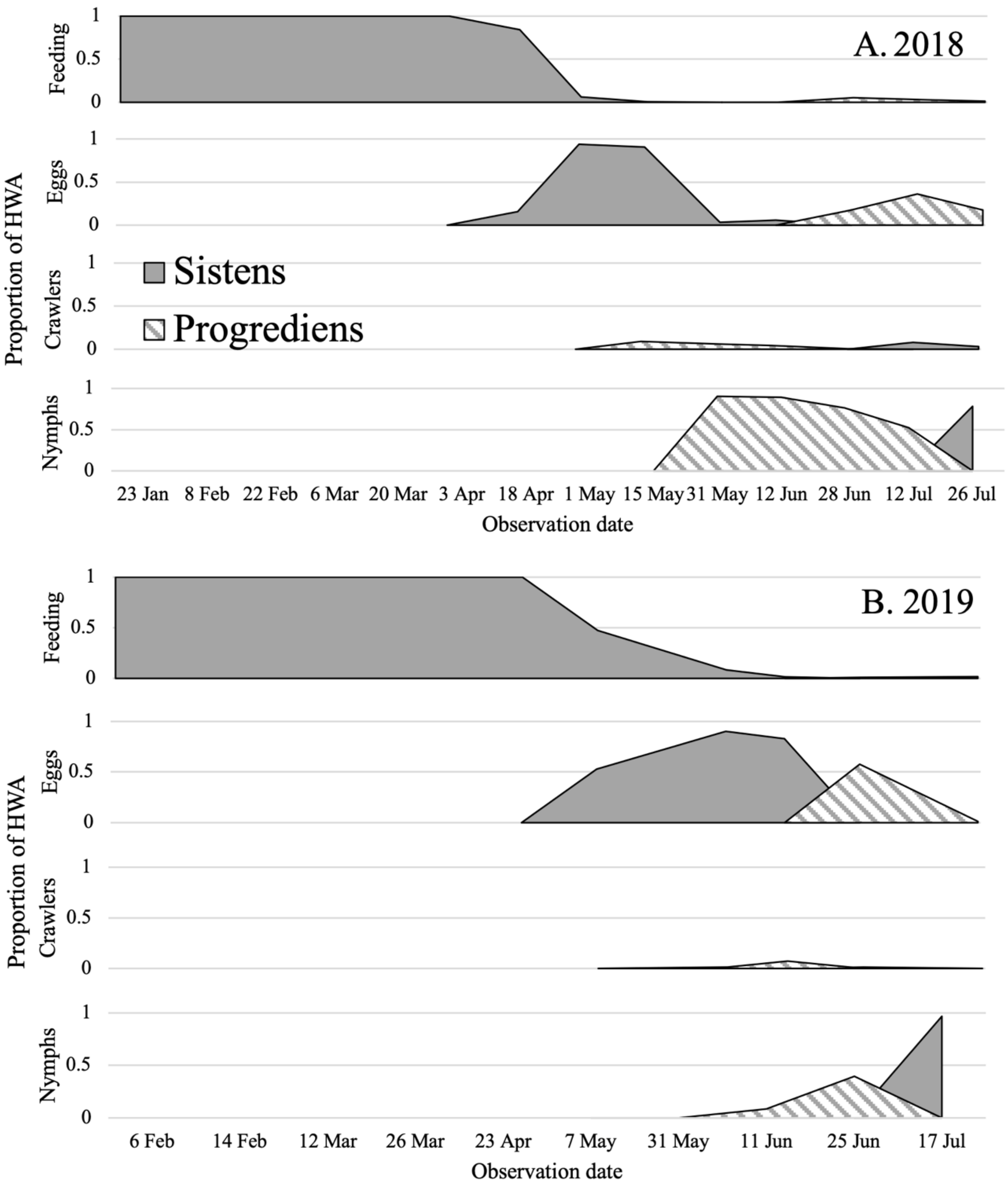

Figure 1.1. Proportion of hemlock woolly adelgids recorded by life stage on 12 hemlock shoots collected at two-week intervals at the Norton site in (A) 2018 and (B) 2019.

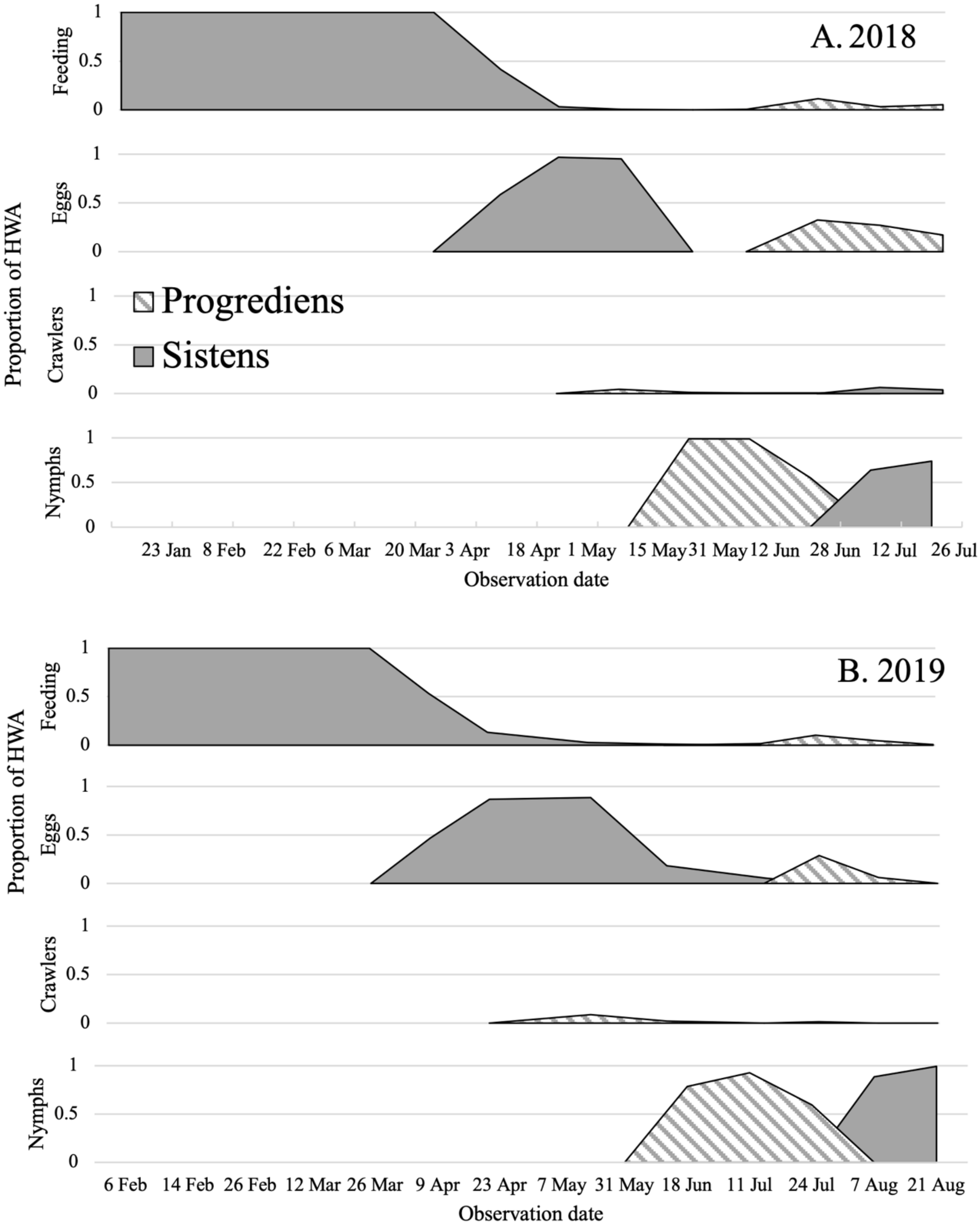

Figure 1.2. Proportion of hemlock woolly adelgid recorded by life stages on 12 hemlock shoots collected at two-week intervals at the Holland site in (A) 2018 and (B) 2019.

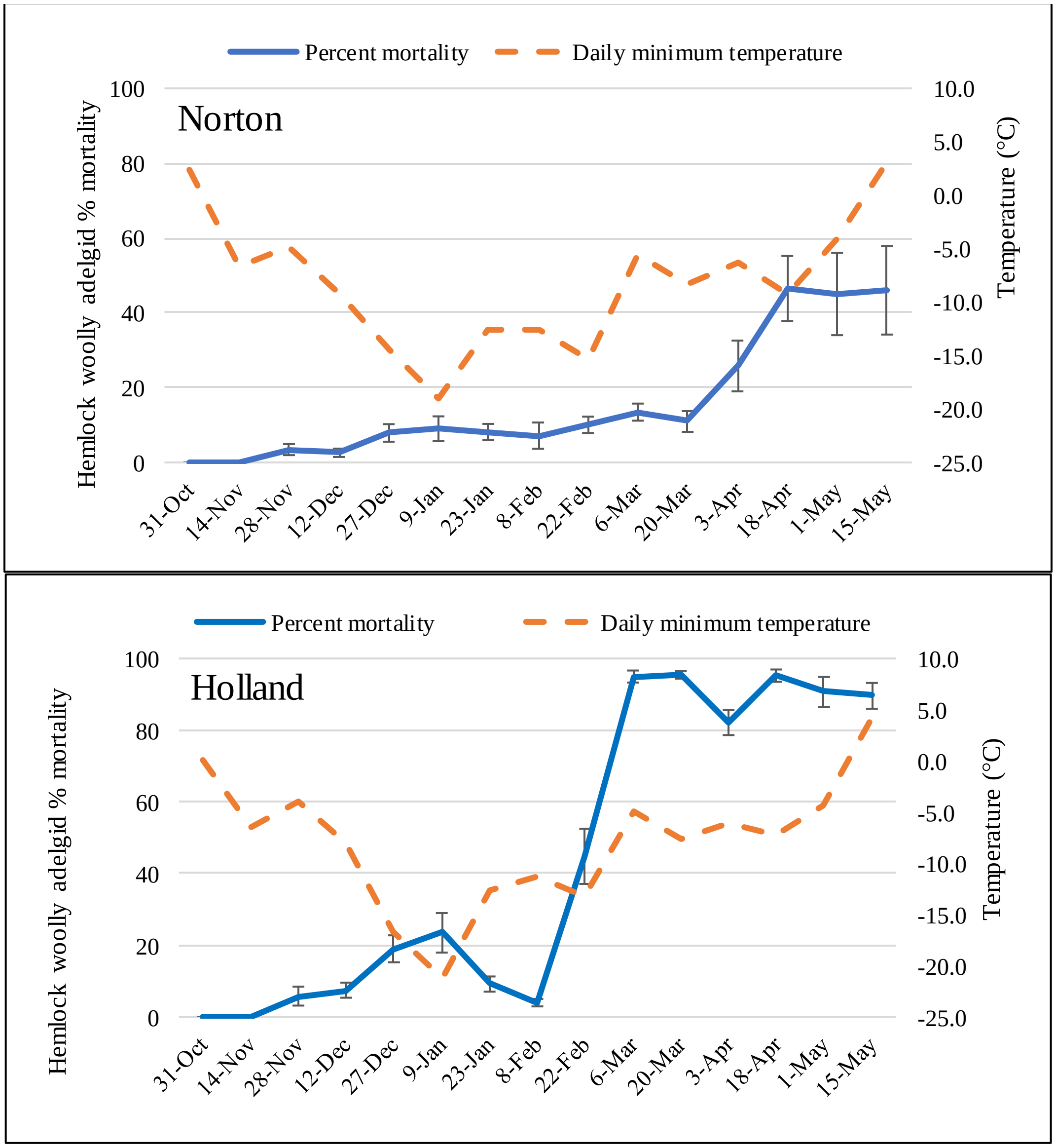

Figure 1.3. Daily minimum ambient temperatures and mean (±SE) percentage of hemlock woolly adelgids that were dead on hemlock shoots collected at two-week intervals between 31 October 2017 and 15 May 2018 from infested trees at the Norton and Holland sites. Twelve shoots were collected from each of two trees per sampling date.

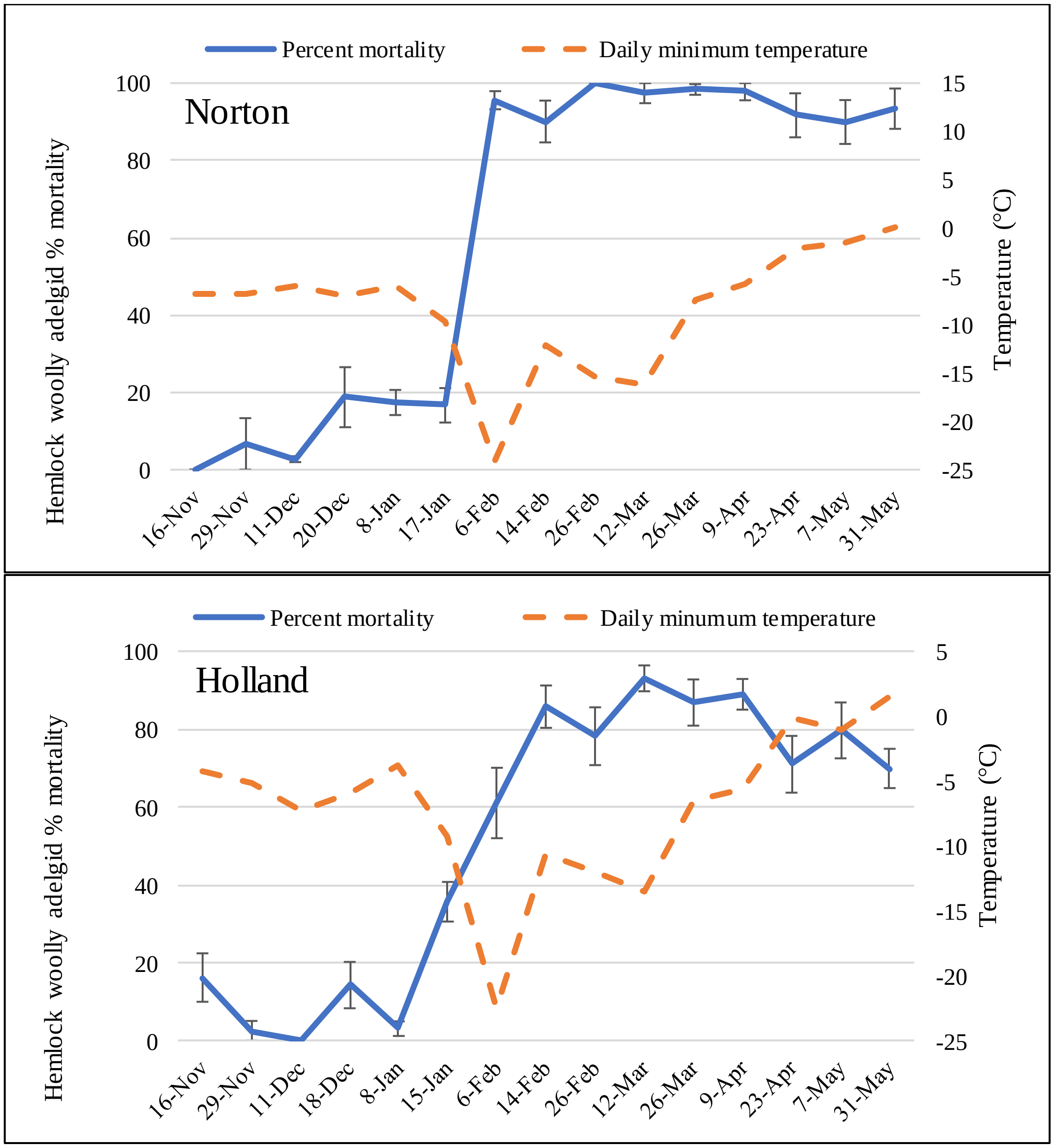

Figure 1.4. Daily minimum ambient temperatures and mean (±SE) percentage of hemlock woolly adelgids that were dead on hemlock shoots collected at two-week intervals between 16 November 2018 and 31 May 2019 from infested trees at the Norton and Holland sites. Twelve shoots were collected from each of two trees per sampling date.

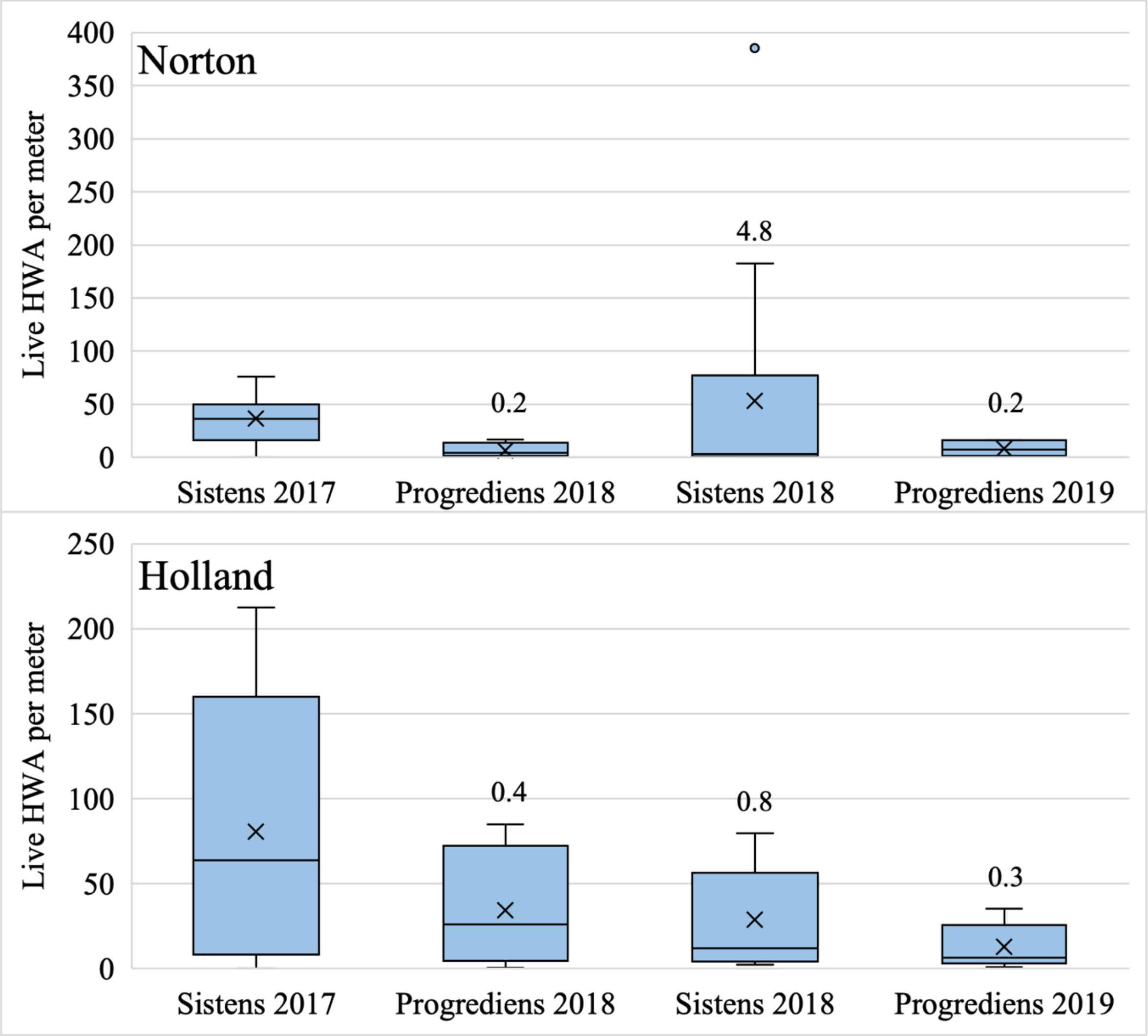

Figure 1.5. Density of actively feeding sistens (October to May) and progrediens (June to July) standardized per meter of foliage-bearing shoot collected in 2017-2018 and 2018-2019 at the Norton and Holland sites. Horizontal lines in the box represent the 1st quartile (bottom of box), median, and 3rd quartile (top of box), and X represents the mean. Vertical lines represent the distance to the minimum and maximum values. Estimated population growth rates (each generation’s total live HWA per m divided by the preceding generation’s total live per m) appear above each box plot.

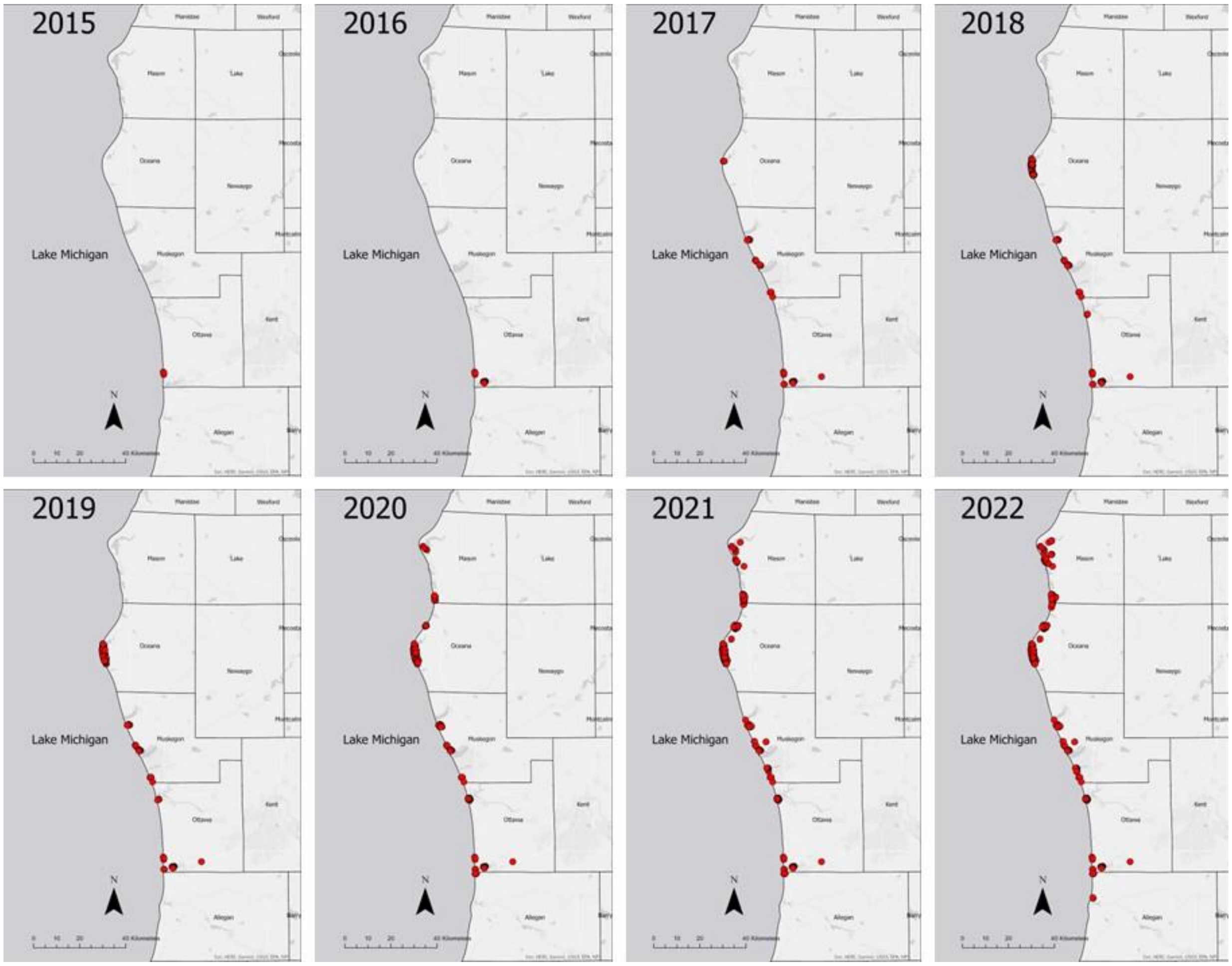

Figure 1.6. Confirmed HWA detections in Michigan from 2015 to 2022 from surveys conducted by Michigan Department of Natural Resources and West Michigan Cooperative Invasive Species Management Area field staff.

CHAPTER 2: EFFICACY AND PERSISTENCE OF IMIDACLOPRID AND DINOTEFURAN APPLIED FOR HEMLOCK WOOLLY ADELGID CONTROL AND POST-TREATMENT HEMLOCK RECOVERY

Introduction

Hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand), an invasive insect native to Japan, was first detected in the eastern United States in Virginia in 1951 (Stoetzel 2002) and is currently established in 20 states and the Canadian provinces of Ontario and Nova Scotia (CFIA 2023, USDA Forest Service 2023). Eastern hemlock, *Tsuga canadensis* (L. Carriere) and Carolina hemlock, *Tsuga caroliniana* (Engelm.), a threatened species that occurs in areas in the southern Appalachian Mountains, are highly vulnerable to HWA (Havill et al. 2016, McClure et al. 2001). Across much of its native range, eastern hemlock is considered an ecological foundation species, with strong influences on soil and aquatic pH, nutrient cycling and other processes (Ellison et al. 2005a, Martin and Goebel 2013). Dense canopies of hemlocks provide critical habitat and shelter for birds and many mammals (Ward et al. 2004), and deer preferentially browse hemlock seedlings and saplings (Frelich and Lorimer 1985). In North America, natural spread of HWA occurs when eggs or crawlers (first instars) are transported by wind or on mammals or birds (McClure et al. 2001). Human transport of infested material, particularly nursery trees, resulted in long distance spread of HWA (Havill et al. 2016). Some states impose external quarantines to prevent HWA introductions, but HWA is not regulated at a federal level (MDARD 2020).

In North America, HWA reproduces parthenogenetically (Havill et al. 2016) and completes two generations annually (Gray and Salom 1996, Havill and Foottit 2007, Havill et al. 2016, McClure 1989). Sistens hatch from eggs in mid- to late-summer and first instar crawlers, the only mobile life stage, move to the base of hemlock needles and enter a period of aestivation, or summer dormancy (Havill et al. 2016, McClure 1989). Sistens begin feeding in fall and continue through the winter, completing four instars before maturing and laying eggs in late winter or spring (Gray and Salom 1996, Havill et al. 2016, McClure 1989). These eggs hatch within a few weeks into progrediens (McClure 1989, McClure et al. 2001). Progrediens, which do not aestivate, go through the same life stages as sistens but mature more quickly and lay eggs in early to mid-summer, which hatch within a few days into sistens (McClure 1989, McClure et al. 2001). Observations in Connecticut indicated HWA sistens laid an average (±SE) of 49 ± 6 eggs per female compared to 22 ± 4 eggs per progrediens female (McClure 1989).

Adelgid crawlers settle near the base of needles on current-year hemlock shoots, insert their long stylet and feed by sucking nutrients from xylem ray parenchyma cells (Young et al. 1995). Feeding HWA secrete white waxy filaments that shield them from predators and desiccation, resulting in a “woolly” appearance (McCullough 2015). Feeding depletes carbon stores in infested trees, causing needles to drop, buds to die and canopies to thin (Domec et al. 2013, McClure 1991, Young et al. 1995). Most infested trees eventually die within 2 to 10 years of colonization (McClure et al. 2001, Ward et al. 2004). Previous research showed that light availability (i.e., sun exposure) improved vigor of hemlocks trees infested by HWA, either by increasing carbon stores (Brantley et al. 2017, Miniat et al. 2020) or perhaps because crawlers were negatively affected by high levels of sunlight (Hickin and Preisser 2015, Mayfield and Jetton 2020).

Systemic neonicotinoid insecticides are widely used in the eastern U.S. to protect individual trees, forests, and watersheds from mortality caused by HWA (Benton et al. 2015, 2016, Coots et al. 2013, Cowles et al. 2005, Cowles et al. 2006, Dilling et al. 2010, Eisenback et al. 2014). Neonicotinoids have the same mode and site of action as nicotine, affecting postsynaptic nicotinic acetylcholine receptors (nAChRs) in the nervous system (Thany 2010). However, in contrast to nicotine, neonicotinoids are more selective to insect acetylcholine receptors than to mammalian receptors, resulting in lower toxicity to humans and other mammals (Thany 2010). Systemic neonicotinoids are typically applied to the base of trees then translocated in xylem to the canopy (Mota-Sanchez et al. 2009, Tanis et al. 2012). Advantages of systemic insecticide products compared with cover sprays include minimal problems related to drift, less environmental contamination (tree injection vs soil drench), fewer effects on non-target species, and control of insects feeding in tall trees or protected by physical barriers such as foliage or bark (Gill et al. 1999, Herms et al. 2019).

Imidacloprid, the first neonicotinoid insecticide, is widely used to control numerous landscape and forest pests (Sheets 2010). It is the most common insecticide used for HWA control (Benton et al. 2015, Coots et al. 2013, Cowles 2009, Vose et al. 2013), typically applied as a soil drench or soil injection (Cowles et al. 2006, Benton et al. 2016). It is also applied via trunk injection for HWA and numerous sap-feeding and phloem- or woodboring insects (Cowles et al. 2006, Doccola et al. 2007. Herms et al. 2019), or as a basal trunk spray (McCullough 2017, Whitmore 2014). Imidacloprid has relatively low water solubility, which results in slow uptake and translocation, especially in conifers that transport water through small diameter tracheids relative to the larger water conducting vessels of hardwoods (Sperry et al. 2006). Applications provide at least 5, and up to 7, years of HWA control (McCullough 2017, Whitmore 2014). Some secondary metabolites of imidacloprid, particularly, olefin, have insecticidal properties and are thought to contribute to the relatively long persistence of HWA control (Benton et al. 2015, Cowles 2009).

Studies to evaluate imidacloprid efficacy or persistence on HWA have involved different application methods, products, and rates; results are variable. Eisenback et al. 2015 reported that effective HWA control following imidacloprid trunk or soil applications was not observed until 2-3 years post-treatment, while Coots et al. 2013 reported imidacloprid soil injections controlled HWA within three months. Efficacy and persistence of trunk-injected imidacloprid is of particular interest, in part because of advances in injection technology that substantially reduced labor and time required for this application method. Moreover, because insecticide is contained within trees, trunk injections are not limited by labels that restrict the amount of active ingredient that can be applied per acre per year. This allows large numbers of trees in a site to be treated in a single visit, an important consideration in forested setting. Basal trunk sprays of imidacloprid are even less common than trunk injections and little information on the efficacy of this application is available.

Dinotefuran, a newer neonicotinoid, is 80 times more water soluble than imidacloprid (Valent 2014), facilitating more rapid uptake and translocation through xylem tissue compared with imidacloprid. Dinotefuran products are commonly applied as a basal trunk spray (Herms et al. 2019, McCullough et al. 2011), but also via trunk injection or soil application (Valent 2014). Dinotefuran, however, is likely less persistent in hemlocks than imidacloprid due to a lack of toxic metabolites, necessitating more frequent re-application (Whitmore 2014). Some states allow dinotefuran to be combined with imidacloprid as a tank mix and applied as basal trunk sprays under a FIFRA Section 2(ee) exception, providing both rapid and persistent HWA control (McCullough 2017, Whitmore 2014).

Established infestations of HWA were first detected in Michigan in 2015 in localized sites near Lake Michigan in two southwestern counties (MDARD 2016). By February 2023, Detection surveys identified infestations in four additional counties, all within approximately 25 km of Lake Michigan (MDNR 2023). More than 170 million hemlocks grow in Michigan forests (USDA Forest Service 2020), providing an array of ecological services, particularly in northern Lower Michigan and the Upper Peninsula. Hemlocks are further key for maintaining dune structure along the Lake Michigan shoreline (West Michigan HWA Taskforce 2019). Given the risk to hemlocks posed by HWA, a multi-agency effort to contain and slow HWA spread was launched in 2017. Infested and adjacent, potentially infested trees are treated with trunk injections of imidacloprid. Dinotefuran is sometimes used, especially when trees are too small to inject. Northernmost HWA infestations are prioritized for treatment. Additionally, many county and local agencies, arborists and private landowners are treating infested hemlocks in areas outside the state HWA program.

In this study, we evaluated rates and persistence of HWA control on infested hemlocks treated with imidacloprid using different application methods or with dinotefuran basal trunk sprays. The canopy condition of treated and untreated trees was monitored and the potential influence of sun exposure or tree DBH diameter on insecticide efficacy and post-treatment tree recovery was assessed.

Materials and Methods

Study Site: The 16 ha Norton site consisted of a semi-permanent campground surrounded by mature forest on steep, sandy dunes immediately adjacent to Lake Michigan near Norton Shores, Muskegon County, Michigan. Overstory composition was dominated by eastern hemlock and American beech (*Fagus grandifolia* (Ehrh.)), with scattered eastern white pine (*Pinus strobus* (L.)), northern red oak (*Quercus rubra* (L.)) and sugar maple (*Acer saccharum* (Marshall)). Infested hemlocks were first detected on this site in 2015. On 21 Sept 2016, 70 infested hemlock trees were selected and GPS coordinates were recorded. Trees were tagged and DBH was measured. Trees were assigned to one of ten blocks, each consisting of seven trees of similar size, condition, HWA infestation level, and location on the site (see methods below). Tree DBH ranged from 4.8 to 69.9 cm with an average of 25.4 ± 1.72 cm.

Pre-Treatment Evaluation of Hemlock Trees: Canopy condition of each tree was visually evaluated and rated on a 1 to 4 scale in September 2016. Trees rated as 1 had healthy canopies with little or no evidence of dead shoot tips, transparency or dead limbs (commonly associated with HWA infestation). A rated of 2 indicated trees had 15-30% canopy transparency and a few dead tips (< 5). Trees rated as 3 had 30-50% canopy transparency and more than five dead tips, while trees rated as 4 had a thin, highly transparent canopy, abundant dead tips, and one or more dead branches or dead sections in the canopy.

Abundance of HWA on each tree was qualitatively rated as low (HWA scarce and confined to shoots on one or two limbs), moderate (HWA present on multiple limbs at varying densities), or high (HWA abundant and present on most limbs).

Insecticide Treatments: Trees within each block were randomly assigned to one of seven treatments: six insecticide applications or an untreated control (Table 2.1). Insecticide applications occurred in fall 2016 / spring 2017 (Table 2.1). Basal trunk sprays of dinotefuran, imidacloprid or the dinotefuran and imidacloprid (dino+imi) tank mix were applied with a 7.5 L garden sprayer and low pressure nozzle to the outer bark, from the base of the trunk to 1.5 m high, ensuring bark around the circumference of the trunk was wet and the proper amount of formulated product was applied. For soil drench applications, litter around the trunk was removed, then formulated product was poured around the base of the tree. Imidacloprid trunk injections were applied using the QuickJet Air® and ArborJet #4 plugs. Slow-release imidacloprid tablets were embedded in soil following label directions. Five of the trees treated with the dinotefuran basal trunk spray in October 2016 were randomly selected and re-treated in September 2018 (Table 1.1).

Evaluation of HWA Mortality: Density of live and dead HWA life stages was periodically evaluated on the 70 trees treated in fall 2016 or spring 2017. Two infested foliage-bearing shoots, each 10-30 cm long (including lateral shoots), were collected from each tree and returned to the MSU Forest Entomology laboratory at approximately four-week intervals from 11 November 2016 to 10 August 2017. Shoot length was measured, then shoots were placed under a microscope and wax was carefully removed to expose the adelgids. Each adelgid was prodded with a pin. If an adelgid exhibited movement or extruded vibrant orange hemolymph when punctured, it was considered alive. If the adelgid had a spongy consistency that resisted puncturing, or extruded sparse, dark hemolymph when punctured, it was recorded as dead. Ovisacs that were off-white or brown, chalky, or containing a heavily desiccated adelgid, were recorded as “old”, representing insect that died the previous season. Once sistens matured and began laying eggs, the number of ovisacs with eggs and number of eggs per ovisac were recorded. When the progrediens generation hatched, the numbers of crawlers and settled nymphs were recorded. Shoot collection was paused during the summer once mature progrediens died and sistens nymphs were aestivating. Similar methods were used to quantify HWA mortality the following season from 14 November 2017 to 24 August 2018. Extremely cold winter temperatures resulted in nearly complete HWA mortality in January 2019, observations of HWA mortality attributable to insecticide treatment ceased after fall 2018 (Chapter 1). Rates of HWA mortality were calculated by dividing the number of dead adelgids by the total number of live plus dead adelgids (current-year only) on each shoot for each sample date.

Post-Treatment Evaluation of Hemlock Condition: Qualitative visual assessments of tree condition were conducted on 28 June and 18 Dec 2018, 5 June and 17 Dec 2019, 17 June and 17 Dec 2020, and 8 June 2021 using the same 1 to 4 scale as the pre-treatment assessments, with a fifth rank added for any dead trees. In June assessments, the abundance of new, current-year foliage was rated from 0 to 3, where 0 indicated no current-year foliage was present, 1 indicated new growth was present on a few shoots, 2 indicated new foliage was present on some shoots but not uniform over the entire tree (often relegated to a certain aspect or section of the tree), and 3 indicated the tree appeared healthy and had abundant new foliage on all or nearly all foliage-bearing branches.

Because canopy condition ranks on 28 June 2018 appeared to be higher when trees were growing in sunny areas than when trees were shaded, tree exposure to sun was evaluated. On 18 Dec 2018, trees were qualitatively rated as 1 if the tree was fully shaded, 2 if partially shaded, and 3 if fully exposed to sun. Subsequently, between 28 August and 5 September 2019, and again from 14 to 16 July 2020, photosynthetically active radiation (PAR) was quantified with a LI-250A light meter (LI-COR Biosciences. Lincoln, NE) in units of µmol. The light meter was held adjacent to shoots on branches at four cardinal aspects, approximately 2 m above ground. Simultaneously, PAR readings were also recorded in open areas receiving full sun. The proportion of full PAR reaching each shoot was calculated by dividing the reading adjacent to a shoot by the simultaneous reading in full sun. Values were averaged for each tree for some analyses. All PAR readings were taken between 10 a.m. and 2 p.m., in either clear or fully overcast conditions.

Photosynthetic potential was measured using the same four shoots used for the PAR reading on each tree. Live needles were separated from shoots, placed into dark adaption clips for 15 minutes, then evaluated with a chlorophyll fluorometer (OS30p+; Opti-Sciences. Hudson, NH). Chlorophyll fluorescence was measured between 15 and 23 October 2019 and 14 to 16 July 2020. Readings consisted of a unitless ratio (Fv/Fm), representing the maximum quantum efficiency of photosystem II. Previous studies of chlorophyll fluorescence in hemlocks established a "stress threshold" value of 0.8 Fv/Fm (Brantley et al. 2016, Miniat et al. 2020). Values below this threshold represent high stress and lower photosynthetic capacity compared to higher values.

Measurements of PAR and needle fluorescence were repeated between 4 January and 3 March 2021, when branches were not shaded by overhead or adjacent hardwood trees. Reading were taken on shoots approximately 3 to 7 m above ground using the same methods described above.

Statistical Analysis: Differences in HWA mortality among insecticide treatments recorded on shoots collected each month in 2016-2017 and 2017-2018 were assessed using one-way ANOVA (PROC GLIMMIX, SAS 9.4) sliced by collection date. Residual plots confirmed normality of mortality data. The Laplace method was applied to estimate error variance for blocking, which was treated as a random effect. Significant differences among treatments identified by ANOVA were separated using the Tukey-Kramer multiple comparison test. Natural mortality of sistens in spring after oviposition and overlap with the subsequent feeding progrediens generation resulted in suspect mortality values in the spring/summer of each year, and so mortality analysis was limited to the sistens generation.

To evaluate hemlock condition over time, a preliminary spaghetti plot of mean canopy condition ranks in the eight visual assessment periods (including the 2016 pre-treatment) was constructed (Figure 2.1). The plot indicated that treatment effects varied with time; hemlock canopy condition increased then declined to stable levels in 2020. Intervals between assessments were evenly spaced, except for the 2016 pre-treatment baseline ranks, which were used as a covariate to facilitate an autoregressive covariance structure for time in our model. Due to the skewed distribution of baseline canopy ranks of “1” (healthy) compared to other ranks, we re-coded the baseline canopy variable as a dichotomous categorical variable (low/high); trees rated as 2 or higher were assigned to a single group. To test for effects of treatment and time on canopy condition rank, we ran a mixed model ANOVA with repeated measures (PROC MIXED, SAS 9.4) while controlling for baseline canopy condition. Because there was no significant interaction between time and treatment, we re-ran the model with fixed effects of time and treatment, and blocks as a random variable. The Satterthwaite approximation for degrees of freedom was applied to ensure model convergence (Schaalje et al. 2002). To find the polynomial term that best described canopy condition over time, we compared models using AIC and determined that including a cubic time term resulted in the best model fit. Significant differences between treatments were further analyzed in pairs with Tukey-Kramer multiple comparison tests.

Extremely cold temperatures in January 2019 killed virtually all HWA on trees in our studies, regardless of treatment. Therefore, a shortened ANCOVA model was used to compare canopy condition ranks from December 2018 with baseline ranks (again treated as a binary and categorical variable) to investigate whether treatment or base canopy ranking up to that point were significant. To evaluate effects of the cold-related HWA mortality in January 2019, we created another ANCOVA model comparing December 2018 with December 2019, using the December 2018 canopy ranks as a covariate. Pair-wise differences among treatments were further analyzed with Tukey-Kramer multiple comparison tests.

Potential effects of sun exposure on canopy condition recorded in June and December 2020 were assessed with boxplots to examine possible interactions between sun/shade ranks and light levels. Because ranks were skewed, trees were divided into two groups; open grown vs. shaded or partially shaded. Similarly, PAR readings were consolidated into shaded trees receiving 0-10% full sun and exposed or partially exposed trees receiving >10% sun. A generalized linear ANOVA (PROC GLM, SAS 9.4) showed that shade and light ranks did not interact, so separate models were created to test effects of each factor individually. To control for differences in canopy condition resulting from insecticide treatments, differences in canopy condition between June 2019 and June 2020, and between December 2019 and December 2020 were used as response variables, with treatment as a main effect. When significant differences were detected, Tukey's HSD for multiple comparison tests was applied.

We similarly evaluated abundance of new foliage growth among treatments. A preliminary spaghetti plot constructed using June observations from 2018 to 2021 showed new growth tended to increase over time to relatively stable levels, regardless of insecticide treatment. An autoregressive covariance structure was again used for time in the analysis because of the even spacing of the sample periods. A mixed model ANOVA with repeated measures (PROC MIXED, SAS 9.4) was conducted using block as a random variable and a quadratic time term to account for the curve over time observed in the spaghetti plot. Interactions of treatment, time, or the quadratic time term were not significant and were dropped from the final model. When significance was detected, pair-wise differences were analyzed with a Tukey-Kramer multiple comparison test.

To test for effects of sun exposure on relative abundance of new, current-year foliage, the same consolidated groupings of shade rank and light group that were used for canopy condition analysis were used in our models. Initial boxplots showed trees were heavily skewed toward the > 10% light group where there was little to no variance in new growth rank. A Wilcoxon signed-rank test was used to compare differences between the two light groups. Within the group of trees exposed to 0-10% light, there was more variation in new growth ranks. A generalized linear model ANOVA (PROC GLM, SAS 9.4) was applied using light/shade ranks as the main effect and new foliage growth as the response variable. When significance was detected, Tukey's HSD was used for comparison between the two groups.

Influence of tree-related variables on chlorophyll fluorescence was assessed for readings taken in June and in December 2020. A t-test (PROC TTEST, SAS 9.4) showed fluorescence values differed between June and December measurements ($t = 84.46$; $df = 132$; $P < 0.0001$). A mixed model ANOVA (PROC MIXED, SAS 9.4) determined the effects of treatment and PAR percentages recorded with the Li-Cor on fluorescence, after model selection using AIC determined those two factors to be the best model fit for our data from among the many physiological variables we recorded. The relationship between exposure to sunlight and chlorophyll fluorescence was further investigated with simple linear regression using all fluorescence and PAR values (PROC REG, SAS 9.4).

Reinfestation of trees by HWA post-treatment was examined using HWA visual assessments recorded from December 2019 to June 2021. This period encompassed the mortality of HWA on all trees caused by extreme cold temperatures in January 2019. Rebounding HWA populations were expected to be greater on trees with less persistent insecticide treatments. Differences between the June 2021 and December 2019 HWA ranks were calculated and used as the response variable for analysis. A generalized linear model ANOVA (PROC GLM, SAS 9.4) with treatment as the main effect was conducted and Tukey's HSD was applied when significance was detected.

Results

Pre-treatment HWA Infestation and Hemlock Canopy Condition: We visually assessed levels of HWA infestation and canopy condition of the 70 hemlocks in September 2016, before insecticides were applied. One tree was lightly infested (rank 1), while 69 trees had a moderate or heavy infestation (rank 2 or 3). On 2 Nov 2016, the first date of shoot collection and HWA analysis, the average density of feeding sistens (secreting wax and forming ovisacs) on control trees was low (Table 2.2), but average sistens nymph densities (immatures that had not yet begun feeding) on control trees ranged from 76 ± 15 to 458 ± 68 per m of foliage-bearing shoot.

While all 70 trees had at least some shoots bearing live HWA, the canopy condition of 61 of the trees were rated as 1 or 2, indicative of a full or nearly full canopy with few or no dead tips. Four trees had some evidence of decline, including more than 2-3 dead tips and were assigned a rating of 3. The remaining five trees were assigned a rating of 4 because canopies were noticeably thin, and several dead tips were present.

Post-Treatment HWA Mortality: During the 2016-2017 winter, average sistens densities on control trees ranged from 4 ± 3 to 194 ± 40 sistens per m of foliage-bearing shoot (Table 2.2). Mortality of HWA sistens between 2 Nov 2016 and 4 April 2017 was affected by insecticide treatment (F = 13.15; df = 4,45; $P < 0.0001$), sampling period (F = 17.09; df = 5,100; $P < 0.0001$), and the interaction of the two factors (F = 6.19, df = 20,100; $P < 0.0001$). On untreated control trees, mortality rates for sistens remained low throughout the winter (Fig. 2.2). In all sampling periods during the 2016-2017 winter, average percent mortality of sistens on control trees was 0 ± 0 to 5 ± 2 %. By 13 January 2017, 99 days post-treatment, differences in sistens mortality among insecticide treatments were apparent (F = 8.34; df = 4,100; $P < 0.0001$) (Fig. 2.2). Sistens mortality was higher on trees treated with basal trunk sprays of dinotefuran alone or mixed with imidacloprid or with the imidacloprid soil drench, while sistens mortality on trees treated with the imidacloprid basal trunk spray and control trees was intermediate. Mortality of sistens on trees treated with dinotefuran alone or dinotefuran plus imidacloprid was higher than mortality on control trees for shoots collected in early Feb, March and April 2017, corresponding to 119, 147 and 180 days post-treatment, respectively (Fig 2.2). While an average of 37 % of sistens on shoots collected in Jan 2017 from trees treated with the imidacloprid soil drench were dead, mortality rates were lower and similar to control trees for shoots collected from these same trees between early Feb and early April (Fig. 2.2). Sistens mortality varied considerably on shoots collected from trees treated with a basal bark spray of imidacloprid, but mortality did not differ from control trees and was consistently lower than mortality of sistens on trees treated with dinotefuran with or without imidacloprid (Fig. 2.2).

During the 2017-2018 winter, the average density of sistens on control trees ranged from 29 ± 19 to 163 ± 88 sistens per meter of foliage-bearing shoot (Table 2.3). Mortality of sistens between 14 Nov 2017 and 6 March 2018 was affected by treatment (F = 30.87; df = 6,47; $P < 0.0001$) and observation date (F = 3.54; df = 4,84; $P = 0.01$), but not by the interaction of the two factors (F = 0.86; df = 24,84; $P = 0.65$). On untreated control trees, mean sistens mortality of around 20 % was observed on 14 Nov 2017, and 9 Jan 2018 (Fig. 2.3). In any sampling period during the 2017-2018 winter, average percent mortality of sistens on control trees was 0 ± 0 to 10 ± 4 %. On 12 Dec 2017, HWA mortality on trees treated with either dinotefuran or imidacloprid, except for the slow-release imidacloprid tablets, was higher ($P < 0.01$) than on untreated control trees (Figure 2.3). On 9 January 2018, HWA mortality on trees treated with trunk injected imidacloprid did not differ from mortality on control trees. On 8 February and 6 March 2018, however, HWA mortality on trees treated with either dinotefuran or any imidacloprid application, except for the slow-release tablets, was higher than on untreated control trees. Mortality of HWA on trees treated with slow-release imidacloprid tablets (Imi Tab) did not differ from the control trees on any sample date.

By December 2018, infestations on most trees were controlled by insecticides. Of the 70 trees, 49 had no evidence of HWA (rank 0) and 12 trees had light infestations (rank 1). Between December 2019 and June 2021, HWA densities on control trees increased compared to other treatments. Results of Dunnett's test indicated HWA levels differed from the controls for trees in all treatments except for those treated with a dinotefuran basal trunk spray ($P = 0.167$). Light and shade measured in either June or December 2020 had no discernable influence on reinfestation.

Post-Treatment Tree Condition: Although all 70 of the hemlocks were infested with HWA in fall 2016, 87 % of the trees had healthy canopies at that time (rank 1 or 2). When we returned to evaluate tree condition in June 2018 this percentage had declined to 59 %, and by December 2018, 19 to 26 months after insecticides were applied, condition of most hemlocks had deteriorated considerably and only 26 % of trees had healthy canopies (Figure 2.1). At this time, over half the trees had poor canopies (rank 4) and one tree that was in poor condition in fall 2016 had died. There was little change in canopies between December 2018 and June 2019 (Fig. 2.1) except that many trees produced new buds and current-year needles were beginning to elongate. Of the 69 live trees, 69% had new buds on half or more of the shoots (ranks 2 or 3), while buds were scarce or absent on the remaining trees. Of the 30 trees treated with basal trunk sprays of either dinotefuran, imidacloprid or the dino+imi tank mix, 27 trees were producing current-year needles (rank 2 or 3). In contrast, two trees of the ten trees treated with the slow-release imidacloprid tablets produced some current-year needles (rank 2) but the other eight trees had few or no new buds. Control trees were evenly split, with five trees producing scarce to no current-year needles (rank 0 or 1), and five trees with some or abundant presence of current-year needles (rank 2 or 3). When we returned to evaluate trees in December 2019, most trees treated with imidacloprid or dinotefuran appeared to be in good canopy condition, with the exception of trees treated with the slow-release imidacloprid tablets (imi tab) and the untreated controls (Figure 2.1).

Across years, abundance of new, current-year foliage was lowest in June 2018 when 35 trees exhibited little or no new growth (rank 0 or 1), while many or even most shoots were producing new buds and foliage on the other 35 trees (rank 2 or 3). In June 2021, 58 trees had abundant new growth while we saw few or no new buds on the other trees. Results from a mixed ANOVA model with repeated measures showed that insecticide treatment (F = 4.26; df = 6,258; *P* = 0.0004), observation period (time) (F = 14.90; df = 1,258; *P* = 0.0001), and the quadratic time term (F = 4.26; df = 1,258; *P* = 0.04) significantly affected new growth ranks. Only trees treated with dinotefuran basal trunk sprays were rated higher than control trees (*P* = 0.03). Trees treated with dinotefuran, dino+imi, and imidacloprid basal trunk sprays all had more new growth than trees treated with imidacloprid slow-release tablets ($P < 0.05$). All other treatments did not significantly differ from each other.

A mixed ANOVA model with repeated measures showed that insecticide treatment, observation date, along with quadratic and time (observation date) significantly affected canopy condition ratings (Table 2.4) and effectively represented the decline and recovery of trees over time. Observation date and insecticide treatment did not interact, indicating the pattern of declining, followed by improving, canopy condition was consistent across insecticide treatments.

Canopy condition of trees treated with a basal trunk spray of dinotefuran alone was rated higher than trees treated with the imidacloprid slow-release tablets and untreated controls ($P < 0.0001$). Similarly, trees treated with the tank mix of dinotefuran plus imidacloprid also differed from trees treated with imidacloprid tablets and controls ($P < 0.0001$). Trees treated with a trunk injection of imidacloprid had higher canopy condition ratings than trees treated with the imidacloprid tablets ($P = 0.0479$), but not higher than controls. Canopy condition of other trees treated with an imidacloprid soil drench or basal trunk spray did not differ from trees in the other treatments or each other.

Neither pre-treatment baseline ranks for canopy condition (F = 0.01; df = 1,52; $P = 0.9$) nor insecticide treatment (F = 1.58; df = 6,52; $P = 0.1$) affected canopy condition in December 2018, indicating that trees had not begun showing signs of recovery before the January 2019 site-wide HWA mortality due to cold temperatures (Chapter 1). An ANCOVA model for December 2019 canopy ranks run with December 2018 ranks and treatment as covariates, however, showed a significant effect of treatment (F = 3.07; df = 6,52; $P = 0.01$) and baseline effect of December 2018 canopy condition (F = 26.58; df = 1,52; $P < 0.0001$). Mean canopy condition of trees treated with the dinotefuran and imidacloprid tank mix treatment differed from controls and from trees treated with the imidacloprid slow-release tablets ($P < 0.05$), while trees in other treatments did not differ significantly from each other or the controls.

Roughly half of the 70 trees (49 %) were either partially or fully shaded (rank 2 or 3) while the others were open-grown and exposed to full or nearly full sun (rank 1). Across all 70 trees, the proportion of photosynthetically active radiation (PAR) reaching trees in June 2020 averaged 8 ± 1 % of the full sun value, ranging from 0.02 to 37 % for individual trees. In December 2020 after hardwood trees dropped their leaves, proportion of PAR reaching shoots on the hemlocks averaged 13 ± 1 % and ranged from 1 to 41 %. We analyzed trees grouped by sun exposure ($\leq$ 10% vs > 10% full sun) to evaluate the response of canopy condition between June 2019 and 2020 and between December 2019 and 2020. Results indicated shade positively affected changes in canopy condition in both June (F = 15.08; df = 1; $P < 0.0003$) and December 2020 (F = 29.36; df = 1; $P < 0.0001$), while insecticide treatment and the interaction were not significant. Production of buds and current-year needles also appeared to positively respond to shade. Results from a generalized linear model showed that within the 0 – 10% full sun group, trees rated as part or fully shaded had significantly higher new growth ranks than those rated as open grown (F = 12.27, $P = 0.001$).

In June 2020, chlorophyll fluorescence averaged 0.82 ± 0.002 and ranged from 0.77 to 0.85 Fv/Fm across all insecticide treatments, while in December 2020, values averaged 0.15 ± 0.01 and ranged from 0.05 to 0.37 Fv/Fm across all treatments. Chlorophyll fluorescence was significantly lower in December 2020 than in June 2020 (t = 84.46; df = 132; $P < 0.0001$). A mixed model ANOVA showed that treatment (F = 3.77; df = 6,57; $P = 0.0031$) and the average proportion of PAR received by trees (F = 27.14; df = 1,57; $P < 0.0001$) significantly affected June fluorescence values. Trees treated with dinotefuran or a mix of dinotefuran and imidacloprid had significantly higher needle fluorescence values than control trees, but needle fluorescence on trees treated with imidacloprid alone did not differ from the controls or from each other. Linear regression showed that PAR explained 19% of the variation in chlorophyll fluorescence values recorded in June 2020 (Figure 2.4) but there was no linear relationship in December 2020 ($P = 0.77$).

Discussion

We monitored sistens mortality after insecticide treatments from November 2016 to December 2018; most treatments resulted in nearly complete HWA control by December 2018. We intended to continue monitoring mortality in 2019, but lethal winter temperatures of -20° C caused complete HWA mortality on all trees in 19 January 2019 (Chapter 1), so that comparisons among treated and control trees were no longer possible. We continued to monitor tree health variables, starting in the summer of 2018, when new needles were seen expanding on many trees.

Both the dinotefuran standalone and dino+imi basal trunk sprays provided rapid control of HWA compared to imidacloprid alone. The dinotefuran treatments controlled HWA within three months post-treatment, while the fastest imidacloprid treatment in our study, the trunk injection, effectively controlled HWA seven months post-treatment. While the tracheids of conifers restrict the speed of systemic insecticides compared to larger hardwood vessels (Sperry et al. 2006), the high water-solubility of dinotefuran allows for quicker translocation and subsequent HWA control than imidacloprid (Faulkenberry et al. 2012, Joseph et al. 2011). Dinotefuran-treated trees only experienced feeding by the 2016-2017 sistens before nearly complete control was observed on 2 March 2017, 147 days post-treatment. In contrast, trees treated in October 2016 with imidacloprid sustained HWA feeding by the entire sistens generation, the spring 2017 progrediens generation, and two months of the fall 2017 sistens generation before high HWA mortality was observed. Dinotefuran's speedy control was reflected in healthier canopies compared to trees treated with only imidacloprid. For high-value forest trees or ornamental trees in landscapes, it may be appropriate to use applications of dinotefuran to ensure as little damage from HWA feeding presents itself as possible, despite the high cost of the product relative to imidacloprid products.

Soil drench applications of imidacloprid for HWA control have been well studied (Benton et al. 2015, Cowles et al. 2006, Dilling et al. 2010, Faulkenberry 2008, Joseph et al. 2011), so the effective control was expected. Our study used a rate of 0.5 to 0.7 g a.i per cm DBH, which resulted in over 50 % HWA mortality at 404 days post-treatment (13 months), and 100 % mortality at 490 days post-treatment (16 months). Rates in previous studies ranged from 0.4 to 0.8 g a.i per cm DBH, and variable results of control have been reported (Benton et al. 2015, Cowles et al. 2006, Dilling et al. 2010, Faulkenberry 2008, Joseph et al. 2011). For example, one study using a rate of 0.8 g a.i per cm DBH reported significant control in 10 months post-treatment (Joseph et al. 2011), while another study used a lower rate of 0.4 g a.i per cm DBH and saw significant control of HWA 9 months post-treatment compared to untreated trees (Cowles et al. 2006). Despite further confirmation of the effective HWA control that soil drenches provide, the use of soil drenches in a forest setting in Michigan is hampered by the proximity of many hemlocks in Michigan to Lake Michigan and other waterways amid a growing concern from the public about insecticide use near water sources.

Basal trunk spray applications of imidacloprid were as effective in our study at controlling HWA as other imidacloprid application methods. Trunk sprays can be made with backpack or low-pressure garden sprayers which are readily available and especially well-suited for small diameter trees that may be difficult to effectively trunk inject. The Mallet®2F T&O product applied at the 0.67g a.i per cm DBH rate in our study costs approximately $0.54 per cm DBH. Tank mixing with dinotefuran could provide a combo of speed and persistence of control, however the cost of dinotefuran in addition to the cost of imidacloprid, as well hitting acre per year label limits much more quickly, makes this technique more suited to saving small numbers of high-value trees rather than broad-scale management in forests.

Trunk injections can have a high initial cost, especially with the Quik-jet Air used in our study currently retailing for $2,599. However, trunk injections remain within tree tissue for the life of the product, and so are not restricted by per-acre insecticide label limits, which can make them practical for protecting hemlock-dense forested areas. Applying the Ima-Jet® product at our rate of 1.6 ml (0.8 g a.i) costs approximately $0.47 per cm DBH, only slightly less expensive than basal trunk sprays. Trunk injections in our study were applied in April 2017 due to limited resources, six months after the basal trunk spray and soil drench imidacloprid applications, yet they caused high HWA mortality at the same time (207 days post-treatment) as the other imidacloprid treatments, aside from the ineffective slow-release tablets. Previous studies on trunk injections used Mauget capsules, typically applied at a rate of 0.6 g a.i per cm DBH (Cowles et al. 2006, Dilling et al. 2010, Eisenback et al. 2014), though there has been some research on the Viper, Wedgle, and Tree I.V. injection systems which all similarly used lower rates of active ingredient than our study (Cowles et al. 2006, Doccola et al. 2007). In studies with Mauget capsules, HWA control was not seen until 13 months, and sometimes up to 2 years post-treatment (Cowles et al. 2006, Eisenback et al. 2014). Mauget capsules are a micro-injection system that utilize low pressure, which results in minimal injury to trees in contrast to macro-injection systems, like the one used in our study, which utilizes larger plugs and higher pressure which can potentially damage the tree (Tanis and McCullough 2016). This higher pressure, in addition to the higher rate we used, however, may account for the speed of HWA control using our trunk injection methods when compared to previous studies.

The slow-release imidacloprid tablets did not provide any HWA control on our study trees. A prior residue analysis study that used the same slow-release tablet product on hemlock trees detected levels of imidacloprid in their samples high enough for HWA control at 12 months post-treatment (Coots et al. 2013). The slow-release tablet application was the only method that was not formulated with water, and we believe that the sandy soils that characterized the dunes at our study site meant that the product did not properly dissolve.

Between September 2016 (pre-treatment) and December 2018, condition of all trees declined regardless of pre-treatment condition or insecticide treatment. Winter mortality of HWA on all trees, including untreated controls, occurred in January 2019. Between December 2018 and December 2019, canopy condition ranks in 2018 predicted 2019 ranks, presumably reflecting this uniform control of HWA on all trees while they steadily recovered. There were also no significant differences in new growth production among trees treated with the effective imidacloprid treatments and the untreated controls, which is also likely due to the HWA control that winter mortality provided.

Canopy condition rating did not differ between shaded trees exposed to ≤10% of full sun, and those receiving at least 10% of full PAR. Production of buds and new needles, however, was positively related to light availability, consistent with previous research (Brantley et al. 2017, Hickin and Preisser 2015, Miniat et al. 2020). This discrepancy between canopy condition and new growth responses could be explained by the fact that our new growth ranks were given independently of overall canopy condition. On trees treated with imidacloprid, for example, HWA feeding caused tree condition to decline before the insects were killed. After HWA were controlled, trees treated with imidacloprid and exposed to full or mostly full sunlight produced new buds.

Chlorophyll fluorescence can be used as an indicator of leaf-level stress (Brantley et al. 2017, Miniat et al. 2020), and values indicative of stress will be below the “stress threshold” level of 0.8 Fv/Fm (Miniat et al. 2020). Chlorophyll fluorescence in June 2020 was highest on trees treated with dinotefuran which supports the healthier canopy condition ratings and higher levels of new growth also observed on trees treated with dinotefuran. Values in December 2020 in our study were extremely low, with control trees averaging 0.16 ± 0.03 Fv/Fm. Brantley et al. (2017) noted low values relative to their summer measurements between 0.55 and 0.6 Fv/Fm during their March sample period, so the low values we recorded likely reflected lower photosynthetic activity occurring in the winter. Overall, trees treated with dinotefuran had HWA infestations controlled quickly, and so indicators of tree health such as chlorophyll fluorescence were subsequently higher on those trees than on trees treated with imidacloprid.

Our study found that most imidacloprid and dinotefuran treatments were equally effective at controlling HWA. The optimal treatment strategy, therefore, depends upon the situation and needs of the landowner or manager. Dinotefuran treatments worked quickly and can protect the health and appearance of trees as evidenced in our study with higher canopy condition ratings and higher chlorophyll fluorescence measurements on trees treated with dinotefuran. Insecticide label limits on acreage per year applications, however, can be hit quickly with trunk sprays or soil drenches and so dinotefuran is most useful for high-value trees or trees with heavy infestations that must be controlled quickly. Imidacloprid treatments would be best for trees in relatively good condition that can handle feeding by HWA until the treatments are able to become effective. All application methods aside from the slow-release tablet were effective at controlling HWA, however our results suggested that the trunk injection may have worked more quickly than other imidacloprid treatments, since it was applied 6 months later but provided the same level of control the following fall. Trunk injections are also a good option for management of high numbers of trees in a forest setting, due to the lack of acreage per year label limits for the method.

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Table 2.1. Insecticide and product names, application methods, dates and rates used to treat 70 hemlock woolly adelgid infested hemlock trees in 2016 and 2017.

Treatment	No. of trees	Mean ± SE DBH	Product	Date of application	Rate per cm DBH[1]	Active ingredient per cm DBH
Imidacloprid						
Basal trunk spray	10	25.5 ± 3.6	Mallet®2F T&O	6 Oct 2016	23.7 ml[2]	0.7 g
Soil drench	10	24.8 ± 3.8	Mallet®2F T&O	6 Oct 2016	0.08 oz DBH < 38.1 cm 0.12 oz DBH >38.1 cm	0.5 g DBH < 38.1 cm 0.7 g DBH >38.1 cm
Trunk injection	10	25.8 ± 4.3	Ima-Jet®	21 April 2017	1.6 ml[3]	0.8 g
Slow-release tablet	10	25.5 ± 3.9	CoreTect®	17 May 2017	1.2 tablets[3]	0.6 g
Dinotefuran						
Basal trunk spray (1 spray)	5	28.0 ± 4.3	Safari®20SG	6 Oct 2016	23.7 ml[2]	0.7 g
Basal trunk spray (2 sprays)	5	19.3 ± 2.9	Safari®20SG	6 Oct 2016 + 31 Oct 2018	23.7 ml[2]	0.7 g
Dinotefuran + Imidacloprid						
Basal trunk spray	10	27.6 ± 4.0	Safari®20SG + Mallet®2F T&O	6 Oct 2016	23.7 ml[2]	0.5 g dinotefuran + 0.7 g imidacloprid
Control	10	24.9 ± 3.9	None	Fall 2016	---	---

[1] Diameter at breast height [2]Formulated product; [3] Standalone product

Table 2.2. Mean ± SE densities of live sistens observed on eastern hemlock trees treated in October 2016 (n = 10 trees per treatment), from shoots sampled approximately monthly from 2 November 2016 to 4 April 2017.

	Sisten density (sistens m^{-1})					
	2016		2017			
	2 Nov	22 Nov	13 Jan	2 Feb	2 March	4 April
Control	4 ± 3	169 ± 88	194 ± 40	158 ± 82	153 ± 73	116 ± 35
Dino Spray	272 ± 158	193 ± 85	80 ± 40	32 ± 20	0 ± 0	30 ± 18
Dino+Imi Spray	1 ± 1	37 ± 6	43 ± 16	15 ± 8	16 ± 12	11 ± 7
Imi Spray	445 ± 102	52 ± 34	140 ± 44	218 ± 119	122 ± 34	119 ± 56
Imi Soil	7 ± 1	172 ± 43	138 ± 37	49 ± 42	86 ± 36	65 ± 33

Table 2.3. Mean ± SE densities of live sistens per meter of foliage observed on eastern hemlock trees treated in October 2016 or April/May 2017 (n = 10 trees per treatment), from shoots sampled approximately monthly from 14 November 2017 to 6 March 2018.

	Sisten density (sistens m^{-1})				
	2017		2018		
	14 Nov	12 Dec	9 Jan	8 Feb	6 March
Control	115 ± 84	29 ± 19	99 ± 40	52 ± 18	163 ± 88
Dino Spray	35 ± 24	0 ± 0	0 ± 0	0 ± 0	3 ± 3
Dino+Imi Spray	2 ± 2	0 ± 0	0 ± 0	0 ± 0	2 ± 2
Imi Spray	26 ± 17	25 ± 25	21 ± 21	0 ± 0	57 ± 51
Imi Soil	45 ± 38	4 ± 4	1 ± 1	0 ± 0	1 ± 1
Imi Tablet	136 ± 114	74 ± 37	129 ± 84	97 ± 61	83 ± 55
Imi Trunk	61 ± 57	19 ± 18	10 ± 6	1 ± 1	1 ± 1

Table 2.4. Results from a type III ANOVA with repeated measures for a mixed effects model to assess effects of insecticide treatment, observation period (Time), and baseline pre-treatment canopy condition on the canopy condition of 70 trees treated in October 2016 or April/May 2017 with various applications of imidacloprid, dinotefuran, or a mix of the two. Interactions were not significant and were excluded from this final model.

Effect	df	F value	*P* value
Treatment	6, 102	6.74	**<.0001**
Time	1, 437	74.61	**<.0001**
Time x Time[1]	1, 440	78.53	**<.0001**
Time x Time x Time[1]	1, 437	70.16	**<.0001**
Baseline Canopy Group[2]	1, 112	0.24	0.6282

[1] Quadratic and cubic time terms were added to model to account for the rise and fall of canopy condition rankings over time.

[2] Pre-treatment canopy rankings from September 2016 were treated as a binary categorical variable; healthy vs. moderately healthy.

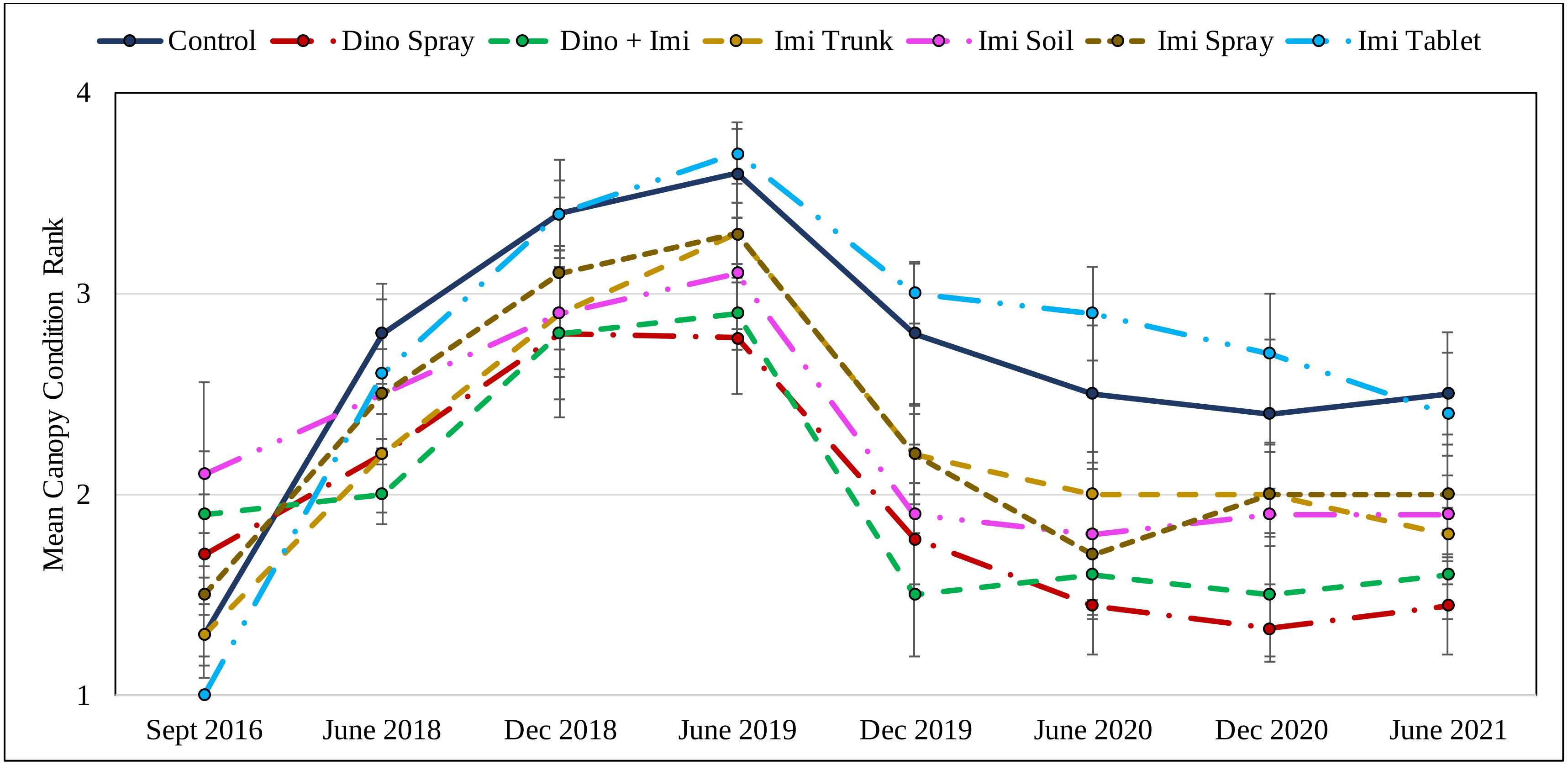

Figure 2.1. Mean canopy condition rating (or score) for trees treated in Fall 2016 or Spring 2017 with a dinotefuran (dino) basal trunk spray, dinotefuran + imidacloprid (imi) basal trunk spray, an imidacloprid basal trunk spray, a soil drench of imidacloprid, an imidacloprid trunk injection, or an imidacloprid slow-release tablet application by treatment over time (n = 10 trees per treatment). Trees were evaluated before insecticide treatments were applied in September 2016, then at six-month intervals from June 2018 to December 2021. Healthy canopies were rated as 1, while poor canopies were rated as 4.

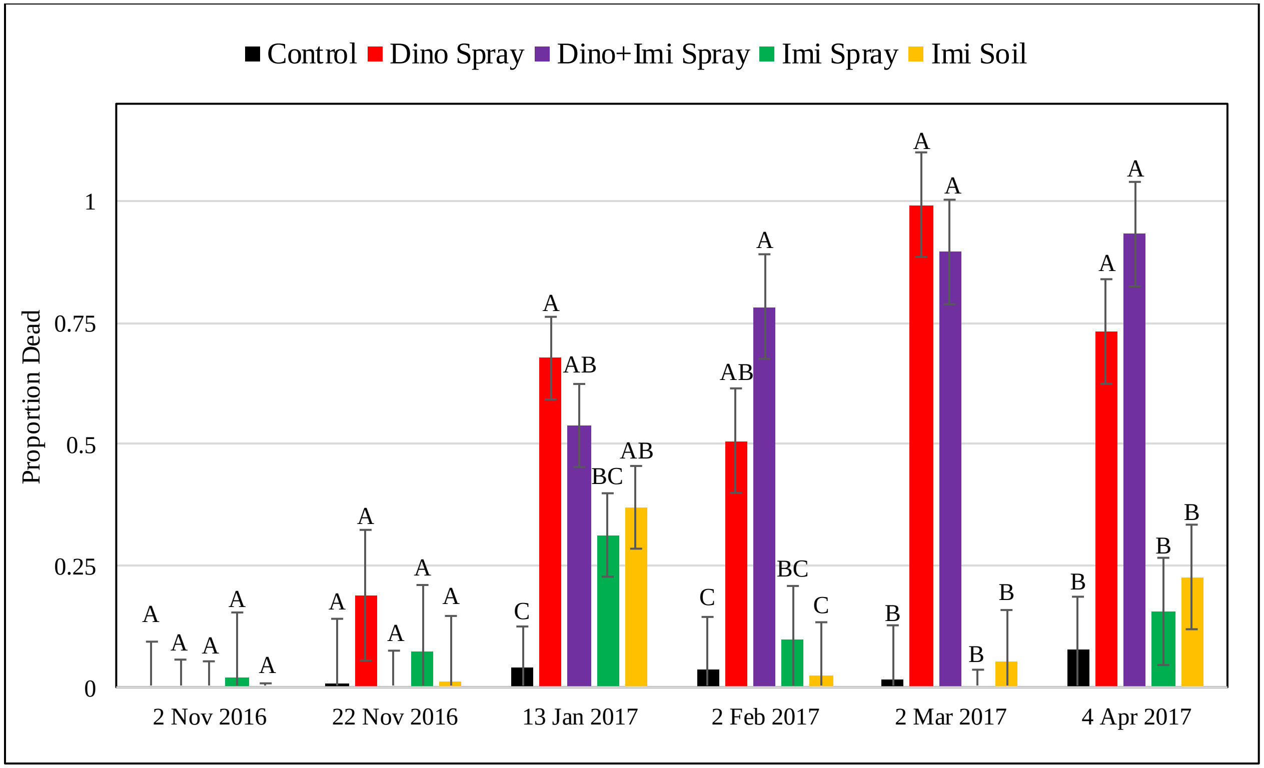

Figure 2.2. Mean (± SE) proportion of hemlock woolly adelgid sistens that were dead on shoots collected periodically from 2 Nov 2016 to 4 Apr 2017 from trees treated with a dinotefuran (dino) basal trunk spray, dinotefuran + imidacloprid (imi) basal trunk spray, an imidacloprid basal trunk spray, or a soil drench of imidacloprid on 6 Oct 2016 and untreated controls. Letters above bars denote significant differences among treatments for each sample date (n = 10 trees per treatment).

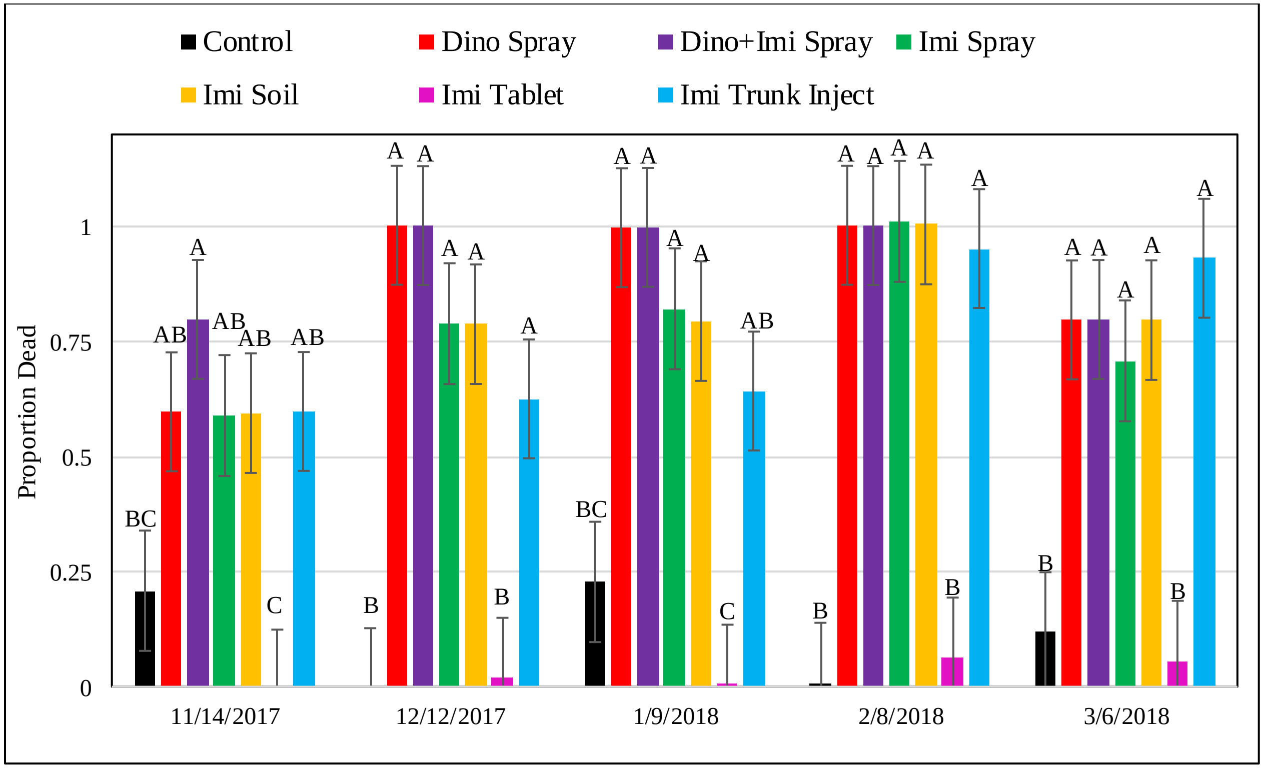

Figure 2.3. Mean (± SE) proportion of hemlock woolly adelgid sistens that were dead on shoots collected periodically from 14 Nov 2017 to 6 March 2018 from trees treated with a dinotefuran (dino) basal trunk spray, dinotefuran + imidacloprid (imi) basal trunk spray, an imidacloprid basal trunk spray, or a soil drench of imidacloprid on 6 Oct 2016, an imidacloprid trunk injection on 21 April 2017, an imidacloprid slow-release tablet application on 17 May 2017, and untreated controls. Letters above bars denote significant differences among treatments for each sample date (n = 10 trees per treatment).

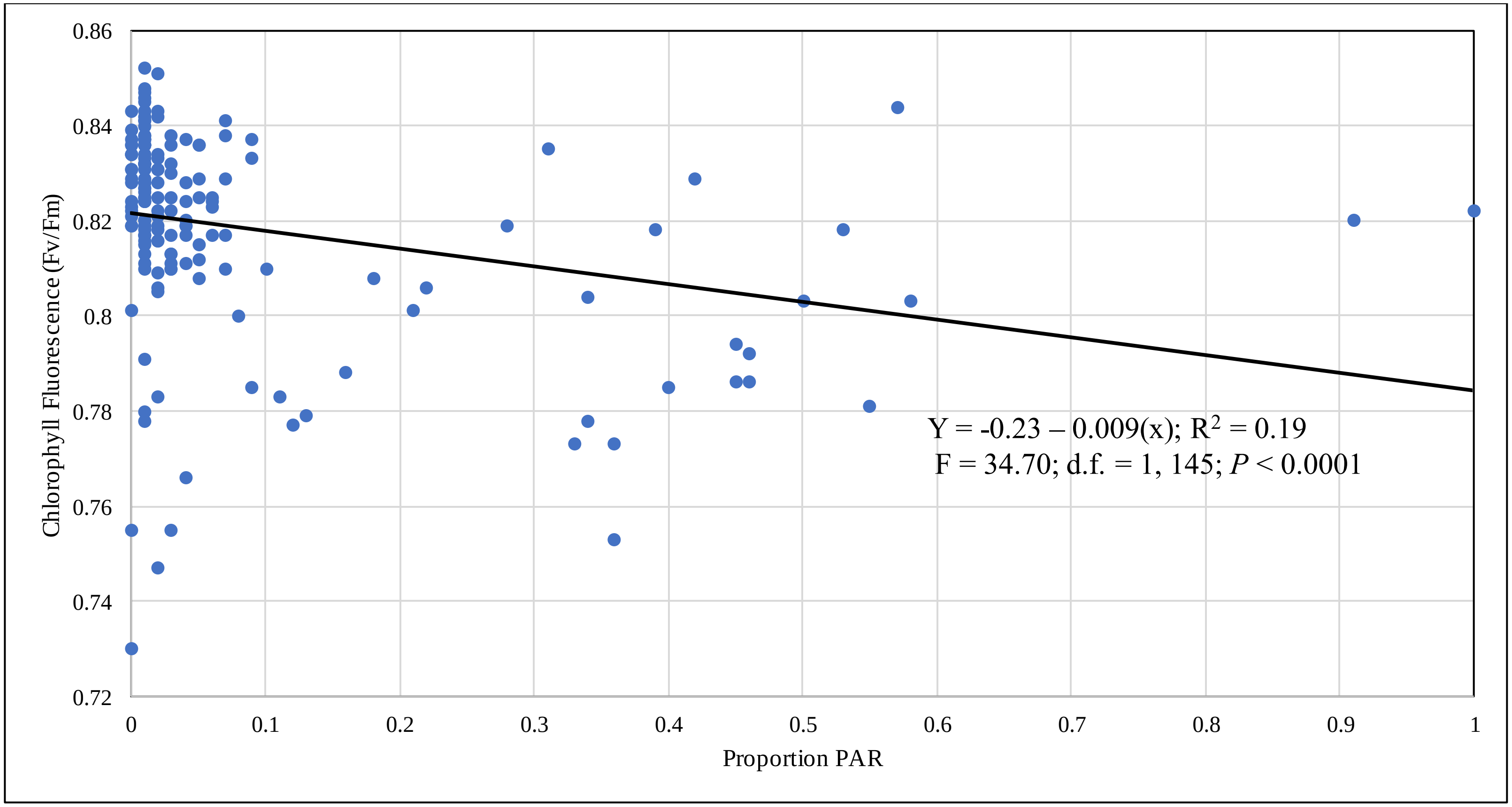

Figure 2.4. Linear relationship between chlorophyll fluorescence and proportion of photosynthetically active radiation (PAR) reaching shoots measured in June 2020 on 70 trees treated with insecticides in Fall 2016 or Spring 2017.

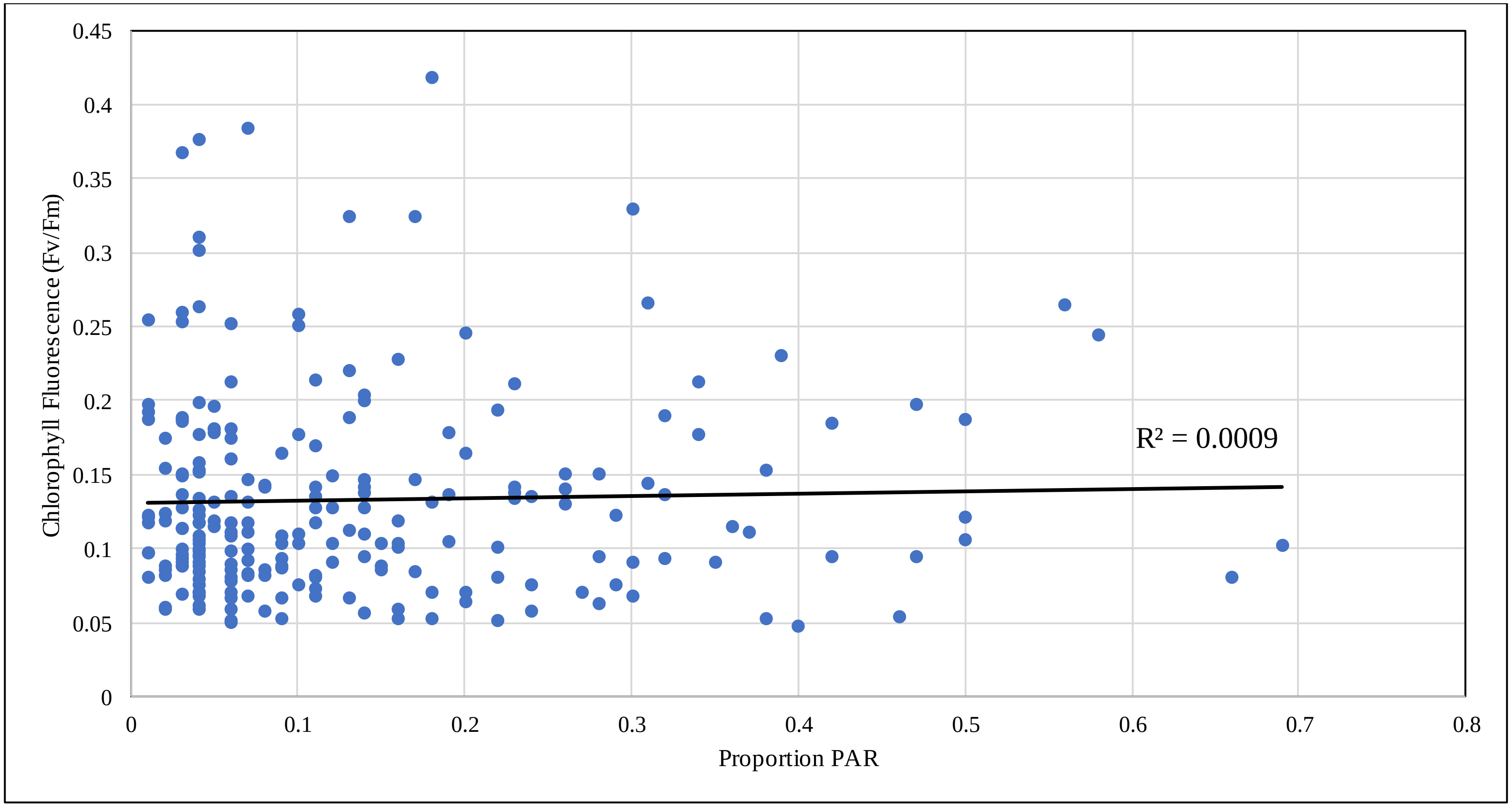

Figure 2.5. Linear relationship between mean chlorophyll fluorescence and mean proportion of photosynthetically active radiation (PAR) reaching shoots measured in December 2020 on 70 trees treated with insecticides in Fall 2016 or Spring 2017.

CHAPTER 3: PERSISTENCE OF IMIDACLOPRID AND DINOTEFURAN RESIDUES APPLIED TO CONTROL HEMLOCK WOOLY ADELGID ON EASTERN HEMLOCK ANALYZED BY TWO METHODS

Introduction

Systemic insecticides are increasingly used to protect trees from pests due to the many benefits that the insecticides offer in comparison to traditional cover sprays. Systemic insecticides are delivered directly into and contained within the treated tree, greatly reducing risks associated with insecticide drift, non-target organisms and applicator exposure in comparison with cover sprays (Gill et al. 1999, Herms et al. 2019). Systemic insecticides, which are transported from roots or base of trees to the canopy through the water conducting xylem elements (Mota-Sanchez et al. 2009, Tanis et al. 2012), also facilitate control of pests feeding in tall trees, under bark or protected by foliage.

Several application methods are available for systemic insecticides. Soil applications require uptake by roots and can include soil drenches (i.e., pouring formulated product around the tree base) or soil injections to place the product beneath herbaceous vegetation (Benton et al. 2016, Cowles et al. 2006). Another option involves dissolvable tablets typically placed into soil near the canopy drip line (Herms et al. 2019, McCullough 2017). Several tools have been devised for injecting insecticides into the conducting sapwood, typically at evenly spaced intervals around the base of the trunk (Cowles et al. 2006, Doccola et al. 2007). Some systems directly introduce insecticide into the drilled holes while others inject the product through a membrane in plastic plugs embedded into drilled holes (Cowles et al. 2006, Doccola et al. 2007, Herms et al. 2019). A third option, basal trunk sprays, involves applying formulated product to the outer bark of the lower 1.5 m of a tree trunk using a low-pressure sprayer to limit any drift.

Insecticide moves through the bark and into the xylem for subsequent translocation (Herms et al. 2019, McCullough 2017).

Translocation rates of systemic insecticides vary considerably depending on product chemistry, tree physiology, and the vascular system in the tree. For example, imidacloprid and dinotefuran are both systemic neonicotinoids, but dinotefuran is 80 times more water-soluble than imidacloprid, so it moves more rapidly through the vascular system to the canopy trees. Given its high solubility, dinotefuran is often applied as a basal trunk spray, although it can also be applied to soil or via trunk injection (Herms et al. 2019, Valent 2014). Insecticide application rates are typically based on tree diameter at breast height (DBH), along with application method and insecticide concentration. Translocation rates differ substantially between hardwood and coniferous trees, reflecting xylem physiology. Relatively narrow tracheids in conifers conduct water, including systemic insecticides, relatively slowly compared to large diameter xylem vessels in hardwoods (Sperry et al. 2006). Regardless of product or tree species, the vascular system must be healthy enough to effectively transport insecticide into the canopy where pests are feeding for a treatment to be effective.

Insecticide persistence within trees, i.e., how long the product remains toxic, also depends on insecticide chemistry and tree physiology. Imidacloprid is typically more persistent than dinotefuran (Faulkenberry et al. 2012, Joseph et al. 2011), which may in part reflect the relatively slow translocation of imidacloprid from the base of trees to the canopy (Tanis et al. 2012). Additionally, imidacloprid breaks down into secondary metabolites like imidacloprid-olefin, which also has insecticidal activity and can extend the duration of pest control (Benton et al. 2015, Coots 2012, Cowles and Lagalante 2009). However, one year after application, foliar imidacloprid residues in *Fraxinus* spp. trees dropped substantially (Mota-Sanchez et al. 2009, Tanis et al. 2012) and were too low to effectively control emerald ash borer (*Agrilus planipennis* Fairmaire) (McCullough et al. 2011, McCullough et al. 2019).

Imidacloprid has long been used to protect eastern hemlock and Carolina hemlock (*Tsuga caroliniana*) from the invasive hemlock woolly adelgid (HWA) (*Adelges tsugae* Annand). This sap-feeding insect, native to Japan, was first detected in the eastern U.S. in Virginia in 1951 (Havill et al. 2016). HWA has since become established in at least 20 states where it has killed hundreds of thousands hemlocks in forests and landscapes (McCullough 2017, MDARD 2023). Hemlock woolly adelgid feeds by inserting its long stylet into cells adjacent to the vascular tissue in foliage-bearing shoots. Feeding depletes nutrients leading to a loss of vigor, needle drop, lack of new bud formation, and eventual tree mortality, typically within 10 years of infestation (Havill et al. 2016, McClure et al. 2001). First instar HWA crawlers can be blown in wind or transported on birds or other animals (McClure 1990), but human-assisted dispersal, particularly on hemlock nursery stock, accounts for long distance spread (MDARD 2023).

Numerous studies in eastern states quantified imidacloprid residues in hemlocks to assess insecticide persistence (e.g., Benton et al. 2015, 2016, Cowles et al. 2005, Cowles et al. 2006, Coots et al. 2013, Dilling et al. 2010, Eisenback et al. 2014), but application methods, rates, products, and level of detectable imidacloprid residues varied among the studies. For instance, the rate of active ingredient applied per cm DBH in these studies ranged from 0.04 to 0.6 g. Several studies have assessed residues over time in hemlocks treated with imidacloprid applied to soil (Benton et al. 2015, Coots 2012, Coots et al. 2013, Cowles et al. 2006, Eisenback et al. 2014, Mayfield et al. 2015), but less is known about imidacloprid persistence in trunk-injected trees. In a study where imidacloprid was applied either to soil or via trunk injection, HWA density was reduced on trees treated with soil applications but not on trees that were trunk injected (Cowles et al. 2006). In other studies that involved imidacloprid injected into the trunk with Mauget capsules (J.J. Mauget Co., Arcadia, CA) (0.06 g a.i per cm DBH), residue levels at similar post-treatment time intervals differed (Cowles et al. 2006, Dilling et al. 2010, Eisenback et al. 2014). In one study, imidacloprid residues ranged from 0.02 to 73.2 ppb in sap from hemlock twigs and needles at 24 months post-treatment (Dilling et al. 2010), while another reported concentrations of 120 ± 30 ppb in pulverized shoots collected nearly three years post-treatment (Cowles et al. 2006). A third study pooled data from trees treated with either trunk injection or soil injections, making direct comparisons difficult (Eisenback et al. 2014, Gervais et al. 2010). Relatively few studies involved recently-developed trunk injection technology that utilizes pressurized air tanks, and we are aware of no research assessing residues following basal trunk sprays of imidacloprid.

Less still is known about dinotefuran persistence in hemlocks. The higher cost of dinotefuran products has limited their use in operational HWA management programs. Relatively rapid translocation of dinotefuran compared to imidacloprid in hemlock presumably facilitates faster control of HWA, an important consideration for heavily infested trees (McCullough 2017, Whitmore 2014). Current recommendations suggest trees may require dinotefuran treatment at two-year intervals for effective HWA control (McCullough 2017, Whitmore 2014) but long term studies on dinotefuran persistence in hemlock or other conifers are lacking. In North Carolina, foliar residues in hemlocks treated with either a basal trunk spray or a soil drench of dinotefuran (Faulkenberry 2008) were assessed for 176 days post-application. While helpful, the short duration of the study limits the understanding of dinotefuran persistence.

Insecticide residues in hemlock tissues can quantified using either enzyme-linked immunosorbent assays (ELISA) (e.g., Cowles et al. 2005, Cowles et al. 2006, Dilling et al. 2010, Eisenback et al. 2008) or ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS) (Coots et al 2013, Benton et al. 2015, Faulkenberry 2008). Quantifying residues using ELISA is less expensive than HPLC/MS/MS analysis, facilitating analysis of many samples, while HPLC/MS/MS can be used to quantify secondary metabolites, along with parent material.

Following the first detections of established HWA populations in Michigan in 2015, a large scale, multi-agency project was launched in 2017 to detect and contain localized HWA infestations, with the goal of protecting the extensive hemlock resource in northern Michigan forests. When new HWA infestations are identified, the infested tree and potentially infested hemlocks within a 245 m radius are treated with imidacloprid or dinotefuran (Bedford et al. 2017). Imidacloprid is most commonly applied via trunk injection, an advantageous method for large-scale treatment projects. Because the insecticide is contained within the tree, applications are not limited by label restrictions that apply to annual soil treatments or basal trunk sprays, so all affected trees in an area can be treated at the same time. In the Michigan program, the Quik-Jet Air® device (Arborjet Inc., Woburn, MA) and plugless injection tools are used to maximize efficiency. Basal trunk sprays are used for dinotefuran applications in some areas or occasionally for imidacloprid, typically when small trees that are difficult to inject must be treated. A FIFRA 2(ee) exemption in some states, including Michigan, facilitates imidacloprid application via basal trunk spray (McCullough 2017, Whitmore 2014).

Information about imidacloprid and dinotefuran concentrations over time in hemlocks treated with trunk injection or basal trunk sprays is needed by HWA program managers, arborists and property owners in Michigan and other states. Limited resources must be efficiently allocated to protect hemlocks and associated ecological services, and to anticipate when re-treatment is needed as residue levels wane. To address such issues, we quantified imidacloprid and dinotefuran residues in samples from hemlock trees treated in fall 2016/spring 2017 or in fall 2018/spring 2019 with various application methods. We assessed insecticide residues separately in needles and woody twigs, both to characterize potential toxicity of those tissues to HWA and to gain information about insecticide translocation. A portion of the samples were divided and analyzed with both ELISA and ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS), which quantified the imidacloprid parent compound and two metabolites known to have toxic properties. Insecticide concentrations generated by ELISA and the UPLC/MS/MS were compared in needles and twigs.

Materials and Methods

Study Site - Study 1: The Norton site, a 16 ha site adjacent to Lake Michigan, consisted of a semi-permanent campground surrounded by steep, sandy forested dunes near Norton Shores, Muskegon Co., MI. Overstory composition was dominated by eastern hemlock and American beech (*Fagus grandifolia*), with a minor component of eastern white pine (*Pinus strobus*), northern red oak (*Quercus rubra*) and sugar maple (*Acer saccharum*). Hemlock woolly adelgid was first detected at this site in 2015. On 21 Sept 2016, 70 infested hemlock trees were identified and tagged. Tree DBH and GPS coordinates were recorded. Trees were assigned to ten blocks of seven trees based on DBH and location within the site. Trees ranged in DBH from 4.8 to 69.9 cm with an average of 25.4 ± 1.72 cm.

Study Sites - Study 2: A second study was launched in September 2018. An additional 42 trees at the Norton site were selected and assigned to six blocks of seven trees as in Study 1. Average DBH for these trees was 14.2 ± 0.81 cm and ranged from 4.6 to 26.4 cm DBH. Infested hemlocks were also selected at a second site near Holland, MI, 48 km south of the Norton site, and approximately 6.5 km east of Lake Michigan. This site consisted of a 4 ha, topographically flat woodlot in Ottawa Co., MI with an overstory composition of eastern hemlock, American beech, and eastern white pine. In September 2018, 28 infested hemlock trees were identified, tagged, and DBH was measured. Tree DBH ranged from 12.2 to 39.4 cm and averaged 23.9 ± 1.6 cm. Trees were assigned to four blocks of seven trees, based on DBH and location.

Insecticide Treatments - Study 1: Within each of the ten blocks selected in fall 2016 at the Norton site, trees were randomly assigned to one of six insecticide treatments or left as an untreated control. Imidacloprid was applied via soil drench, basal trunk spray, trunk injection, or slow-release tablets following label directions in fall 2016 or spring 2017 as additional product became available (Table 3.1). Imidacloprid was applied as a soil drench by gently raking litter away from the base of the tree then pouring formulated product around the circumference of the tree base. Slow-release tablets were applied by embedding tablets approximately 7-8 cm under the soil surface, within 25-30 cm of the tree base and evenly spaced around the trunk. Basal trunk sprays were applied to outer bark around the circumference of the trunk using a common garden sprayer and applying formulated product at low pressure from ~1.5 m high down to the tree base until the appropriate dose was applied. Trunk injections of imidacloprid were applied using a QUIK-Jet Air® device and plugs (ArborJet Inc., Woburn, MA) inserted into holes drilled into the base of the trunk at or just above root flares. Dinotefuran was applied as a basal trunk spray, either alone or at a 25 % lower concentration in a tank mix with imidacloprid (dino+imi), in October 2016 (Table 3.1) using methods as described above. Two years later, in October 2018, five of the ten trees treated with dinotefuran basal trunk sprays in fall 2016 were randomly selected and re-treated with the same product and rate (Table 3.1).

Insecticide Treatments - Study 2: In Study 2, we compared fall versus spring applications with the same products, treatment methods and rates using the three blocks of trees selected in fall 2018 at the Norton site and four blocks of trees at the Holland site. Three trees per block at both sites were randomly assigned to be treated with imidacloprid trunk injections, basal trunk sprays of dinotefuran or basal trunk sprays of the dino+imi tank mix in fall 2018 (Table 3.1). These three treatments were chosen due to high and/or speedy HWA mortality observed in a prior insecticide control study (Chapter 2), prompting the desire for further investigation into how treatment timing could influence the persistence of these treatments. These same treatments were repeated with three additional trees in each block at both sites in spring 2019 (Table 3.1). The remaining tree in each blocks was left as an untreated control.

Insecticide Residues: To quantify insecticide residues, a total of four foliage-bearing shoots, each 30 – 40 cm long, were collected from two aspects of the mid and upper canopies of all 140 hemlocks in Study 1 and Study 2 between 20 May and 4 June 2020 (355 to 1337 days post-treatment depending on application date), and again between 1 and 10 December 2020 (550 to 1526 days post-treatment). Shoots from each tree were placed into a paper bag, returned to the MSU Forest Entomology lab, and air-dried on wire shelves for at least two weeks until needles easily detached from woody twigs. Foliage and woody twigs from each tree were separately ground in a commercial blender between 23 June and 21 August 2020. To avoid cross-contamination, personnel changed nitrile gloves between samples, and blenders were tripled rinsed, washed with detergent (LIQUINOX®, Alconox Inc., White Plains, NY) and scrubbed with a bottle brush, then sprayed with 95% ethanol and finally rinsed with DI water to ensure any insecticide residue was removed. After processing, hemlock foliage and twig samples were divided into two portions; one was used for ELISA while the second portion was analyzed with LC/MS. Processed samples were stored in a freezer until analyzed.

Samples were shipped overnight to the USDA APHIS lab in Sandwich, MA on 29 September 2020 and again on 22 February 2021 to quantify concentrations of imidacloprid (Envirologix; Portland, ME, USA) and dinotefuran (FujiFilm/Horiba; Kyoto, Japan) using commercial ELISA kits. A 0.5 g aliquot of ground needle or twig tissue was weighed into a 50 mL plastic centrifuge tube and extracted in 10 mL of pure methanol for 3 hours on a table-top shaker. Sample tubes were spun down in a high-speed centrifuge for 10 min and the supernatant diluted a minimum of 20x, which eliminates potential matrix effects associated with methanol and the kits. Samples were then run on the assay kits according to the manufacturer's instructions.

Samples of needle and woody twigs collected from Study 1 trees in May/June 2020 were also analyzed by the Michigan State University Mass Spectrometry and Metabolomics Core Facility using ultra performance liquid chromatography - tandem mass spectrometer (UPLC-MS/MS), which combines physical separation capabilities of liquid chromatography with mass spectrometry. Concentrations of dinotefuran, total imidacloprid (sum of all compounds), imidacloprid parent compound, and two metabolites, olefin-imidacloprid and 5-hydroxy-imidacloprid were quantified. For the UPLC/MS/MS analysis, approximately 50 mg of ground hemlock tissue was extracted with one mL of acetonitrile: water (8:2, v/v) containing 100 nM imidacloprid-d4 overnight at 10°C. One hundred µL (microliters) of the extract were evaporated in a speed vac and reconstituted in acetonitrile: water (1:9, v/v). Standard calibration curves were constructed using imidacloprid, olefin-imidacloprid, and 5-hydroxy-imidacloprid (Toronto Research Chemicals, Canada) over a range of concentrations from 0 to 2.5 µM with imidacloprid-d4 (Toronto Research Chemicals, Canada) as internal standards. Residues were quantified on a Waters Xevo TQ-XS UPLC/MS/MS (Waters Corporation, Milford, MA). Five µL of each sample were separated using a 7-min LC gradient on a Waters Acquity BEH C18 UPLC column (2.1 x 50 mm, 1.7 µm) with mobile phases consisting of 0.1% (v/v) formic acid in water (solvent A) and acetonitrile (solvent B). The 7 min gradient was: 2% B at 0.00 to 1.00 min, linear gradient to 99% B from 1.00 min to 5.00 min, hold at 99% B from 5.00 to 6.00 min, then return to 2% B from 6.01 to 7.00 min. Flow rate was 0.3 mL per min and the column temperature was held at 40 °C. The ultrahigh performance mass spectrometer was operated in positive electrospray ionization mode and data were collected in multiple reaction monitoring (MRM) channels. Source parameters were: capillary voltage: 1.0 kV; source temperature: 150°C; desolvation temperature: 400°C; desolvation gas flow: 800 L/hour; and cone gas flow: 150 L/hour.

Statistical Analysis: Insecticide residues (ppm), the response variable for all statistical models, were tested for normality with the Shapiro-Wilks test (proc univariate, SAS 9.4) and visually assessed with Q-Q plots. Data were normalized with a log transformation (ln[x+0.02]), to account for presence of zeroes in both the ELISA and UPLC/MS/MS data. Foliage and twig samples from five trees (10 samples total) with abnormally high residue values, indicative of uneven insecticide translocation, were excluded from statistical analyses. For UPLC/MS/MS data, “imidacloprid” refers to the aggregate value of the parent compound plus all metabolites unless otherwise specified.

For Study 1 trees, analysis of covariance (ANCOVA) was used to evaluate the effects of application method and tree DBH on imidacloprid and dinotefuran residues generated by ELISA for twig and needle samples collected in June and December 2020 (PROC GLM, SAS 9.4).

Samples collected in June and December 2020 were analyzed separately. One-way ANOVA (PROC MIXED, SAS 9.4) was used to evaluate whether application method affected imidacloprid, olefin, 5-hydroxy and dinotefuran residues quantified by UPLC/MS/MS in needle and twig samples collected in June 2020. A Tukey-Kramer multiple comparison test was applied if ANOVA results were significant. Blocks were treated as a random variable in analyses. Blocking was removed from analyses when the covariance was zero, indicating blocks had no effect on those models.

For Study 2 trees, treated in 2018/2019, ELISA residue data were not able to be normalized with transformations. A nonparametric alternative to a two-way ANOVA was applied to ranked residue values (PROC RANK, SAS 9.4), with treatment method, season, and the interaction as factors. A chi-square distribution (Wald-type) was used to determine overall model significance (PROC MIXED, SAS 9.4). Samples collected in June and December 2020 were analyzed separately, and if significance was detected, paired t-tests were used to assess differences between residues in foliage and twigs.

Results

Residue Levels - Imidacloprid: Average residues quantified by ELISA for Study 1 trees treated with imidacloprid and sampled in June 2020 ranged from 0.29 ± 0.10 to 4.24 ± 1.23 ppm in needles and 0.08 ± 0.03 to 0.86 ± 0.21 ppm in twigs (Table 3.2). All treatments increased imidacloprid residues in needles relative to the untreated controls (Table 3.3, Figure 3.1). Residues in needles of trees treated with the imidacloprid slow-release tablets were lower than residues in foliage of trees treated with one of the other four imidacloprid application methods (Figure 3.1). In twig samples, residues from trees treated with slow-release tablets did not differ from those in twigs from control trees (Figure 3.1). In December 2020 samples, average imidacloprid residues ranged from 0.62 ± 0.23 to 1.94 ± 0.68 ppm in needles and 0.16 ± 0.06 to 0.97 ± 0.25 ppm in twigs in treated trees (Table 3.2). Differences among iimidacloprid residues in foliage collected in December 2020 were similar to those identified in the June 2020 samples (Figure 3.1). In twig samples, trees treated with a basal trunk spray (tank mix), trunk injection or soil drench had higher residues than trees treated with the slow-release tablets, while residues in twigs from trees treated with the basal trunk spray did not significantly differ from those of any other trees treated with imidacloprid (Figure 3.2). Twig residues from trees treated with slow-release tablets did not differ from residues in twigs from control treess. Tree DBH did not influence twig or needle imidacloprid residues in either June or December 2020 (Table 3.3).

On average, imidacloprid residues quantified with UPLC/MS/MS from Study 1 trees treated with imidacloprid and sampled in June 2020 ranged from 0.52 ± 0.21 to 3.99 ± 1.39 ppm in needles and 0.09 ± 0.03 to 1.27 ± 0.29 ppm in twigs (Table 3.4). Residues in needles were higher in all trees treated with imidacloprid than in untreated controls (Table 3.5, Figure 3.2). Trees treated with slow-release tablets had lower foliar residues than trees treated with any of the other application methods. Trees treated with the dino+imi basal trunk spray or the imidacloprid trunk injection had higher foliar imidacloprid residues than foliage from trees treated with the basal trunk spray of imidacloprid alone (Figure 3.2). In twigs, residues were higher in trees treated with the trunk injection than trees treated with the basal trunk spray (Figure 3.2).

Residues of olefin-imidacloprid, a secondary metabolite, that were quantified with UPLC/MS/MS in Study 1 trees treated with imidacloprid and sampled in June 2020 averaged 0.05 ± 0.02 to 0.35 ± 0.12 ppm in needles and 0.01 ± 0.003 to 0.04 ± 0.01 ppm in twigs (Table 3.4). All treatments except the slow-release tablets had higher olefin- imidacloprid residues in needles than foliage samples from controls. (Figure 3.2). In twigs, only trees treated with the imidacloprid trunk injection had higher olefin residues than the control trees (Figure 3.2).

Residues of the 5-hydroxy-imidacloprid metabolite quantified with UPLC/MS/MS from trees treated with imidacloprid and sampled in June 2020 averaged 0.02 ± 0.01 to 0.13 ± 0.05 ppm in needles and 0.01 ± 0.004 to 0.04 ± 0.01 in twigs (Table 3.4). Needle residues of 5-hydroxy-imidacloprid were higher in treated trees than in control trees, except for trees treated with the slow-release tablets (Figure 3.2). Needle residues of 5-hydroxy-imidacloprid also did not differ between trees treated with the slow-release tablet and basal trunk sprays (Figure 3.2). In twigs, 5-hydroxy-imidacloprid residues from trees treated with the slow-release tablets did not significantly differ from the controls and were significantly lower than twig residues from all other imidacloprid treatments (Figure 3.2).

Linear regressions found strong relationships between imidacloprid quantified by ELISA and total imidacloprid, imidacloprid parent material, olefin, and 5-hydroxy-imidacloprid generated by UPLC/MS/MS for both needles and twigs (Figure 3.3). Foliar imidacloprid residues (including all metabolites) in needles ranged from approximately 0.2 to 5.0 ppm higher than residues from ELISA analysis of the same samples. For twig samples, however, residues from both analyses were closely related.

In Study 2 trees treated with imidacloprid, residues quantified with ELISA in samples collected in June 2020 and averaged 1.59 ± 0.99 to 3.85 ± 1.26 ppm in needles and 0.47 ± 0.23 to 1.30 ± 0.59 ppm in twigs across treatments (Table 3.6). Imidacloprid residues from needles and twigs of treated trees were higher than those of control trees but did not differ from each other (Figure 3.4). Treatment season (fall vs spring) did not affect residues in the June 2020 samples (Table 3.7). In December 2020, average residues in trees treated with imidacloprid ranged from

1.76 ± 0.55 to 6.32 ± 1.50 ppm in needles and 0.61 ± 0.22 to 1.99 ± 0.39 ppm in twigs (Table 3.6). Imidacloprid residues in needle and twig samples were higher than samples from controls but did not differ among imidacloprid treatments (Figure 3.4). The only significant interaction between insecticide treatment (application method) and season was for imidacloprid twig samples collected in December 2020 (Table 3.7).

Residue Levels - Dinotefuran: On average, dinotefuran residues from Study 1 trees sampled in June 2020 and quantified by ELISA ranged from 0.14 ± 0.05 to 0.91 ± 0.59 ppm in needlesand 0.10 ± 0.05 to 0.17 ± 0.05 ppm in twigs(Table 3.2). For samples collected in December 2020, average dinotefuran residues ranged from 0.10 ± 0.06 to 0.82 ± 0.36 ppm in needles, and 0.18 ± 0.09 to 0.42 ± 0.15 ppm in twigs (Table 3.2). Residues did not differ among trees treated with basal trunk sprays (with or without imidacloprid) or controls nor were they affected by tree DBH for samples collected in either June or December (Table 3.3, Figure 3.1).

When dinotefuran in samples from Study 1 trees collected in June 2020 were quantified by UPLC/MS/MS, average values ranged from 0.15 ± 0.07 to 0.67 ± 0.31 ppm in needles, and 0.03 ± 0.01 to 0.08 ± 0.04 ppm in twigs (Table 3.4) and differed among treatments (Table 3.5). Dinotefuran residues in needle samples from Study 1 trees treated with basal trunk sprays were higher than controls (Figure 3.2). Residues in twigs from trees treated with dinotefuran alone or the tank mix in 2016 did not differ from trees re-treated in 2018 or the controls (Figure 3.2). Residues in twigs were higher in trees re-treated with dinotefuran in 2018 than in the controls.

Dinotefuran residues determined by ELISA and UPLC/MS/MS analyses were correlated for foliage but not for twigs (Figure 3.5). Dinotefuran residues in needles quantified by UPLC/MS/MS ranged from 1.51 ppm lower to 0.31 ppm higher than values determined by ELISA, and from 0.37 ppm lower to 0.08 ppm higher in twigs.

For Study 2 trees sampled in June 2020, average dinotefuran residues quantified by ELISA and ranged from 0.71 ± 0.17 to 3.39 ± 0.67 ppm in needles and 0.08 ± 0.04 to 0.24 ± 0.06 ppm in twigs (Table 3.6) and residues in both tissues differed between trees treated in fall 2018 and spring 2019 (Table 3.7). Trees treated with basal trunk sprays of dinotefuran alone had higher needle residues than trees treated with the tank mix of dino+imi (Figure 3.4). In twigs, dinotefuran residues did not differ among treated trees but all were higher than controls(Figure 3.4). In samples of Study 2 trees collected in December 2020, average dinotefuran residues ranged from 0.32 ± 0.09 to 1.69 ± 0.66 ppm in needles and 0.14 ± 0.07 to 0.43 ± 0.29 ppm in twigs (Table 3.6). Dinotefuran residues in needles were higher in treated trees than controls but did not differ from each other (Table 3.7, Figure 3.4). Residues in twigs in December 2020 did not differ from controls or from each other (Figure 3.4).

Discussion

Systemic insecticides, primarily imidacloprid, are essential for protecting ecological services in forests where hemlock trees are relatively abundant and threatened by HWA invasion, as well as amenity hemlocks in landscapes (Lovett et al. 2006, Orwig et al. 2012). No parasitoids attack HWA and despite 20 years of efforts to identify, rear and release insect predators (Kirtane et al. 2022, Preston et al. 2023), there is no evidence that individual or collective efforts of predators can protect trees from HWA-related decline. Determining the persistence and re-treatment interval of insecticides is a key question faced by HWA managers, who must balance operational budgets, availability of labor and product, and label limits, which can dictate which treatment methods are available.

Our results showed that, apart from the slow-release tablets, imidacloprid applied via basal trunk spray, soil drench and trunk injection remained at or above concentrations associated with effective HWA control (>0.3 ppm) in both needles and twigs (Cowles and Lagalante 2009) for at least four years post-treatment. Residue levels were higher in needles than in twigs for all treatments, reflecting the movement of imidacloprid in sap through xylem into the foliage, where a previous study found imidacloprid accumulated in *Fraxinus* trees (Mota-Sanchez et al. 2009). Low residues in trees treated with slow-release tablets may have been caused by the tablets not fully dissolving or, if the tablets did dissolve, the imidacloprid may have leached out of the root zone in the sandy soil that characterized the Norton study site. The insecticide label for the slow-release tablets states that the release of the active ingredient is dependent on adequate moisture, so the sandy soils may have hindered the proper release and absorption of the product (Bayer 2007).

In Study 2, a major goal was to determine if applying insecticides in fall versus spring affected in residues or persistence. Summer applications of systemic insecticides are generally not recommended because of concerns that dry soils could inhibit uptake or translocation in hemlocks (Whitmore 2014). Our results indicate hemlocks can be effectively treated in either spring or fall, which extends opportunities and flexibility for operational programs where labor or budgetary constraints must be considered. Both seasons correspond to typically cool to moderate soil temperatures and moisture conditions that facilitate systemic insecticide uptake. Translocation may be somewhat faster with spring applications as expanding buds and needles act as a strong sink for water and associated compounds moving through the xylem (Mota-Sanchez et al. 2009). Systemic compounds applied in fall may require more time to reach upper canopy shoots , but as buds swell and break in spring, translocation rates presumably increase. Additionally, given that HWA sistens are actively feeding through the winter and spring, while progrediens feed from late spring to mid summer generations, fall or spring applications can be expected to eventually reduce local HWA densities (Doccola et al. 2007).

In Michigan, the multi-agency effort to contain localized HWA infestations relies largely on trunk injections of imidacloprid, along with basal trunk sprays of dinotefuran or dinotefuran mixed with imidacloprid in some situations. Neither trunk injections nor basal trunk sprays of imidacloprid have as well-studied as the imidacloprid soil drenches and injections commonly used in research and operational programs in eastern states (Coots et al. 2013, Cowles et al. 2006, Dilling et al. 2010, Eisenback et al. 2014). We found imidacloprid residues, including secondary metabolites, were similar regardless of whether trees were trunk injected, treated with a basal trunk spray, or with a soil drench. Residues in needle and twig samples collected in both June and December 2020 (up to 1329 days post-treatment) consistently exceeded the laboratory-based 0.3 ppm LC_{50} and the field-based 0.12 ppm LC_{50} reported for HWA (Cowles et al. 2006). Because insecticide is contained within the tree tissues, trunk injections are not limited by the per acre per year restrictions that apply to soil applications and basal trunk sprays (MDARD 2018). This is notably advantageous, particularly for large-scale operational programs where numerous trees in a given area must be treated in a single year. Advances in trunk injection technology, such as the Quik-Jet Air® used in our studies, and plugless injection methods used by many HWA managers in Michigan, further enhance the practicality of these large-scale treatments.

Although application rates for systemic insecticide products are based on tree DBH, in a previous study, tree diameter and phloem area were linearly related for ash (*Fraxinus* spp) trees up to approximately 30 cm DBH, but the relationship was exponential for larger trees (McCullough and Siegert 2007). We hypothesized that the canopy area of hemlocks needing protection from HWA could also vary between trees with similar DBH but varying exposure to sun, different stocking levels or tree vigor. Our results showed, however, that neither imidacloprid nor dinotefuran residues were related to tree diameter, perhaps reflecting the consistent relationship between the area of active xylem and live foliage in hemlock. In some studies, reduced rates for imidacloprid applied to soil provided effective HWA control while enabling managers to treat more trees in a given area at one time (Benton and Cowles 2017, Cowles 2009, Eisenback et al. 2014, Joseph et al. 2011). Whether the duration of imidacloprid toxicity was affected by the reduced rates is not known. Our research results along with the ongoing Michigan HWA program demonstrate the feasibility of effectively and efficiently applying imidacloprid via trunk injection, which effectively avoids annual limits applied to sprays or soil applications.

Analyses by UPLC/MS/MS quantified the olefin and 5-hydroxy imidacloprid metabolites, both of which have insecticidal properties for HWA, and which may be responsible for the length of HWA control seen in hemlocks after treatment with imidacloprid (Coots et al. 2013, Benton et al. 2015). Dose-response assays have reported 80% mortality of HWA at olefin concentrations of 0.007 ppm, and at 5-hydroxy concentrations of 0.142 ppm (Coots 2012). None of the mean residue values of 5-hydroxy detected in June twig samples were at or above the lethal concentration of 0.142 ppm for any application method, and only the needle samples from the dino+imi tank mix had a high enough concentration of 5-hydroxy to be considered lethal to HWA. June 2020 samples from all Study 1 trees that were treated with imidacloprid had average olefin levels above 0.007 ppm, apart from twig samples of trees treated with slow-release tablets. Trees treated with the trunk injection and dino+imi tank mix had the highest concentrations in both needle and twig samples, albeit not significantly higher than the other treatments. The trunk injection was also the only treatment where olefin residue values in twig samples were significantly higher than in twigs from control trees. A previous study on trunk-injected imidacloprid in ash trees found that the accumulation in tissues was relative to the plane of the injection site, with higher concentrations of insecticide detected in samples collected directly above the injection points (Tanis et al. 2012), and so this uneven distribution may be responsible for the higher olefin concentrations we observed in the twig samples from trunk-injected trees. Imidacloprid first breaks down into 5-hydroxy, which then breaks down into olefin (Benton et al. 2015), so it is also possible that olefin residues in the Study 1 trees will continue to accumulate for some time.

Guidelines suggest dinotefuran re-application intervals for HWA control may be no more than 1-2 years (Whitmore 2014, McCullough 2017), but persistence of dinotefuran in hemlocks has received much less attention compared with imidacloprid. Based on results from residue analyses in our study, the two-year period of HWA seems appropriate. While no LC_{50} for HWA control has been determined, dinotefuran levels in trees treated with basal trunk sprays, with or without imidacloprid, were barely detectable at 19 months post-treatment. Trees treated with dinotefuran in spring 2019 had higher dinotefuran levels than the other dinotefuran-treated trees, likely because at the time of sampling in May/June 2020, these treatments would only recently have crossed the one-year mark (355 to 370 days post-treatment depending on the exact sampling date for each tree), and dinotefuran was applied at a 25 % lower rate when mixed with imidacloprid than it was when applied as a standalone spray. While not nearly as persistent as imidacloprid, the value of dinotefuran comes from its quick and effective control, and in a previous study was shown to provide nearly 100 % HWA control at 51 days post-treatment and had significantly higher levels of HWA control at 26-, 51-, and 176-days post-treatment compared to imidacloprid treatments (Faulkenberry et al. 2012). With two generations of HWA feeding each year, this speed of control is essential for protecting high-value trees before needle-drop and other visible decline can occur.

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Table 3.1. Insecticide and product names, application methods, dates and rates used to treat 70 hemlock woolly adelgid infested hemlock trees in Study 1 in 2016 / 2017, and 70 additional trees in Study 2 in 2018 / 2019.

Treatment	No. of trees	Mean ± SE DBH	Product	Date of application	Rate per cm DBH[1]	Active ingredient per cm DBH
Imidacloprid						
Basal trunk spray	10	25.5 ± 3.6	Mallet®2F T&O	6 Oct 2016	23.7 ml[2]	0.7 g
Soil drench	10	24.8 ± 3.8	Mallet®2F T&O	6 Oct 2016	0.08 oz for DBH < 38.1 cm 0.12 oz for DBH >38.1 cm	0.5 g for DBH < 38.1 cm 0.7 g for DBH >38.1 cm
Trunk injection	10	25.8 ± 4.3	Ima-Jet®	21 April 2017	1.6 ml[3]	0.8 g
	10	18.3 ± 3.0		31 Oct 2018		
	10	20.2 ± 2.8		31 May 2019		
Slow-release tablet	10	25.5 ± 3.9	CoreTect®	17 May 2017	1.2 tablets[3]	0.6 g
Dinotefuran						
Basal trunk spray (1 spray)	5	28.0 ± 4.3	Safari®20SG	6 Oct 2016	23.7 ml[2]	0.7 g
	10	18.4 ± 2.4		31 Oct 2018		
	10	18.3 ± 3.1		31 May 2019		
Basal trunk spray (2 sprays)	5	19.3 ± 2.9	Safari®20SG	6 Oct 2016 + 31 Oct 2018[3]	23.7 ml[2]	0.7 g
Dinotefuran + Imidacloprid						
Basal trunk spray	10	27.6 ± 4.0	Safari®20SG + Mallet®2F T&O	6 Oct 2016	23.7 ml[2]	0.5 g dinotefuran + 0.7 g imidacloprid
	10	19.0 ± 2.9		31 Oct 2018		
	10	18.8 ± 2.8		31 May 2019		
Control	10	24.9 ± 3.9	None	Fall 2016	---	---
	10	19.0 ± 3.0	None	Fall 2018	---	---

[1] Diameter at breast height [2]Formulated product; [3] Standalone product

Table 3.2. Mean (± SE) imidacloprid and dinotefuran insecticide residues in needles and twigs from composite samples of hemlock shoots collected from Study 1 trees (n = 10 trees per treatment) in June and December 2020. Residues were generated by enzyme-linked immunosorbent assay (ELISA).

	Treatment	Needles	Twigs
		Mean ± SE (ppm)	Mean ± SE (ppm)
June 2020	Imidacloprid		
	Control	0.03 ± 0.03	0
	Dino + Imi	1.88 ± 0.56	0.75 ± 0.26
	Imi Inject	4.24 ± 1.23	0.86 ± 0.21
	Imi Soil	1.31 ± 0.17	0.43 ± 0.08
	Imi Spray	0.90 ± 0.16	0.26 ± 0.05
	Imi Tablet	0.29 ± 0.10	0.08 ± 0.03
	Dinotefuran		
	Control	0.11 ± 0.04	0.11 ± 0.05
	Dino	0.14 ± 0.05	0.17 ± 0.05
	Dino Re-Treat[1]	0.91 ± 0.59	0.15 ± 0.07
	Dino + Imi	0.34 ± 0.19	0.10 ± 0.05
December 2020	Imidacloprid		
	Control	0	0
	Dino + Imi	1.94 ± 0.68	0.73 ± 0.22
	Imi Inject	1.89 ± 0.54	0.97 ± 0.25
	Imi Soil	1.59 ± 0.35	0.48 ± 0.12
	Imi Spray	1.11 ± 0.19	0.37 ± 0.07
	Imi Tablet	0.62 ± 0.23	0.16 ± 0.06
	Dinotefuran		
	Control	0.08 ± 0.03	0.31 ± 0.11
	Dino	0.10 ± 0.06	0.18 ± 0.09
	Dino Re-Treat	0.82 ± 0.36	0.42 ± 0.15
	Dino + Imi	0.35 ± 0.21	0.31 ± 0.14

[1] Five of 10 trees treated in Oct 2016 were re-treated in Oct 2018

Table 3.3. Results from a type III ANCOVA evaluating the main effects of insecticide treatment method and tree diameter (DBH) on needle and twig insecticide residues in composite samples of hemlock shoots collected from Study 1 trees (n = 10 trees per treatment) in June and December 2020. Residues were generated by enzyme-linked immunosorbent assay (ELISA).

Samples		Treatment Method			Tree Diameter (DBH)		
		d.f.	F Value	Pr>F	d.f.	F Value	Pr>F
June 2020	Imidacloprid						
	Needle	5, 47	25.55	**< 0.0001**	1, 47	3.56	0.0656
	Twigs	5, 44	18.59	**< 0.0001**	1, 44	1.89	0.1762
	Dinotefuran						
	Needle	3, 24	1.3	0.296	1, 24	1.05	0.3152
	Twigs	3, 24	0.84	0.4844	1, 24	0.38	0.5451
December 2020	Imidacloprid						
	Needle	5, 50	21.69	**< 0.0001**	1, 50	0.17	0.685
	Twigs	5, 50	15.49	**< 0.0001**	1, 50	0.63	0.4306
	Dinotefuran						
	Needle	3, 23	2.97	0.0532	1, 23	2.87	0.1036
	Twigs	3, 23	1.07	0.3801	1, 23	3.99	0.0577

Table 3.4. Mean (± SE) imidacloprid and dinotefuran insecticide residues in needles and twigs from composite samples of shoots collected from Study 1 trees (n = 10 trees per treatment). Residues were obtained using ultra-high performance liquid chromatography with tandem mass spectrometry (UPLC/MS/MS) on samples collected in June 2020.

	Treatment	Needles	Twigs
		Mean ± SE (ppm)	Mean ± SE (ppm)
June 2020	Imidacloprid		
	Control	0.004 ± 0.003	0
	Dino + Imi	3.99 ± 1.39	0.84 ± 0.28
	Imi Inject	9.63 ± 2.78	1.27 ± 0.29
	Imi Soil	1.73 ± 0.25	0.48 ± 0.09
	Imi Spray	1.26 ± 0.28	0.26 ± 0.05
	Imi Tablet	0.52 ± 0.21	0.09 ± 0.03
	Olefin		
	Control	0	0
	Dino + Imi	0.35 ± 0.12	0.03 ± 0.01
	Imi Inject	0.16 ± 0.04	0.04 ± 0.01
	Imi Soil	0.13 ± 0.03	0.02 ± 0.01
	Imi Spray	0.07 ± 0.01	0.01 ± 0.003
	Imi Tablet	0.05 ± 0.02	0
	5-hydroxy		
	Control	0	0
	Dino + Imi	0.13 ± 0.05	0.03 ± 0.01
	Imi Inject	0.09 ± 0.02	0.04 ± 0.01
	Imi Soil	0.08 ± 0.01	0.03 ± 0.01
	Imi Spray	0.05 ± 0.01	0.03 ± 0.003
	Imi Tablet	0.02 ± 0.01	0.01 ± 0.004
	Dinotefuran		
	Control	0.01 ± 0.01	0.01 ± 0.003
	Dino	0.15 ± 0.07	0.03 ± 0.01
	Dino Re-Treat[1]	0.67 ± 0.31	0.08 ± 0.04
	Dino + Imi	0.19 ± 0.07	0.04 ± 0.01

[1] Five of 10 trees treated in Oct 2016 were re-treated in Oct 2018

Table 3.5. Results of a type III ANOVA to evaluate effects of insecticide treatment method on insecticide residues in hemlock needles and twigs in composite samples of shoots collected from Study 1 trees (n = 10 trees per treatment). Residues were obtained using ultra-high performance liquid chromatography with tandem mass spectrometry (UPLC/MS/MS) on samples collected in June 2020.

		Treatment d.f.	F Value	Pr>F
June 2020	imidacloprid			
	needle	5, 41	29.86	**< 0.0001**
	twig	5, 50	27.63	**< 0.0001**
	olefin			
	needle	5, 37	10.61	**< 0.0001**
	twig	5, 46	5.05	**0.0009**
	5-hydroxy			
	needle	5, 37	9.94	**< 0.0001**
	twig	5, 37	12.45	**< 0.0001**
	dinotefuran			
	needle	3, 10	15.39	**0.0004**
	twig	3, 10	3.87	**0.0449**

Table 3.6. Mean (± SE) imidacloprid and dinotefuran insecticide residues in needles and twigs from composite samples of shoots collected from Study 2 trees (n = 10 trees per treatment) in June and December 2020. Residues were quantified by enzyme-linked immunosorbent assay (ELISA).

	Treatment	Needles	Twigs
		Mean ± SE (ppm)	Mean ± SE (ppm)
June 2020	Imidacloprid		
	Control	0.05 ± 0.03	0
	Fall D+I	1.93 ± 0.70	0.88 ± 0.26
	Spring D+I	1.59 ± 0.99	0.47 ± 0.23
	Fall Trunk Inject	3.67 ± 0.94	1.28 ± 0.41
	Spring Trunk Inject	3.85 ± 1.26	1.30 ± 0.59
	Dinotefuran		
	Control	0.01 ± 0.01	0.02 ± 0.01
	Fall Dino	0.71 ± 0.17	0.08 ± 0.04
	Spring Dino	3.39 ± 0.67	0.24 ± 0.06
	Fall D+I	0.48 ± 0.16	0.11 ± 0.06
	Spring D+I	1.49 ± 0.47	0.16 ± 0.05
December 2020	Imidacloprid		
	Control	0	0
	Fall D+I	2.59 ± 0.69	0.95 ± 0.22
	Spring D+I	1.76 ± 0.55	0.66 ± 0.16
	Fall Trunk Inject	6.32 ± 1.50	1.99 ± 0.39
	Spring Trunk Inject	3.44 ± 1.23	0.61 ± 0.22
	Dinotefuran		
	Control	0.01 ± 0.01	0.16 ± 0.09
	Fall Dino	0.41 ± 0.16	0.26 ± 0.13
	Spring Dino	1.69 ± 0.66	0.43 ± 0.29
	Fall D+I	0.32 ± 0.09	0.14 ± 0.07
	Spring D+I	0.34 ± 0.11	0.15 ± 0.09

Table 3.7. Type III tests of fixed effects to assess insecticide treatment method, season (fall, spring), and the interaction of insecticide residues in hemlock needle and twig samples from composite samples of shoots collected from Study 2 trees (n = 10 trees per treatment) in June and December 2020. Residues were quantified by enzyme-linked immunosorbent assay (ELISA).

		Treatment			Season			Treatment x Season		
		d.f.	χ^2 Value	Pr > χ^2	d.f.	χ^2 Value	Pr > χ^2	d.f.	χ^2 Value	Pr > χ^2
June 2020	imidacloprid									
	needle	2, 51	30.16	**< 0.0001**	1, 51	1.23	0.2678	2, 51	0.64	0.7273
	twig	2, 51	32.67	**< 0.0001**	1, 51	2.11	0.1459	2, 51	1.06	0.5875
	dinotefuran									
	needle	2, 54	138.83	**< 0.0001**	1, 54	10.14	**0.0014**	2, 54	5.1	0.078
	twig	2, 54	11.17	**0.0038**	1. 54	7.19	**0.0073**	2, 54	4.06	0.1316
December 2020	imidacloprid									
	needle	2, 54	100.93	**< 0.0001**	1, 54	1.29	0.2562	2, 54	1.81	0.4037
	twig	2, 54	127.96	**< 0.0001**	1, 54	17.04	**< 0.0001**	2, 54	14.91	**0.0006**
	dinotefuran									
	needle	2, 54	71.28	**< 0.0001**	1, 54	3.86	**0.0494**	2, 54	3.55	0.1691
	twig	2, 54	0.56	0.7569	1, 54	0.38	0.5381	2, 54	0.29	0.8633

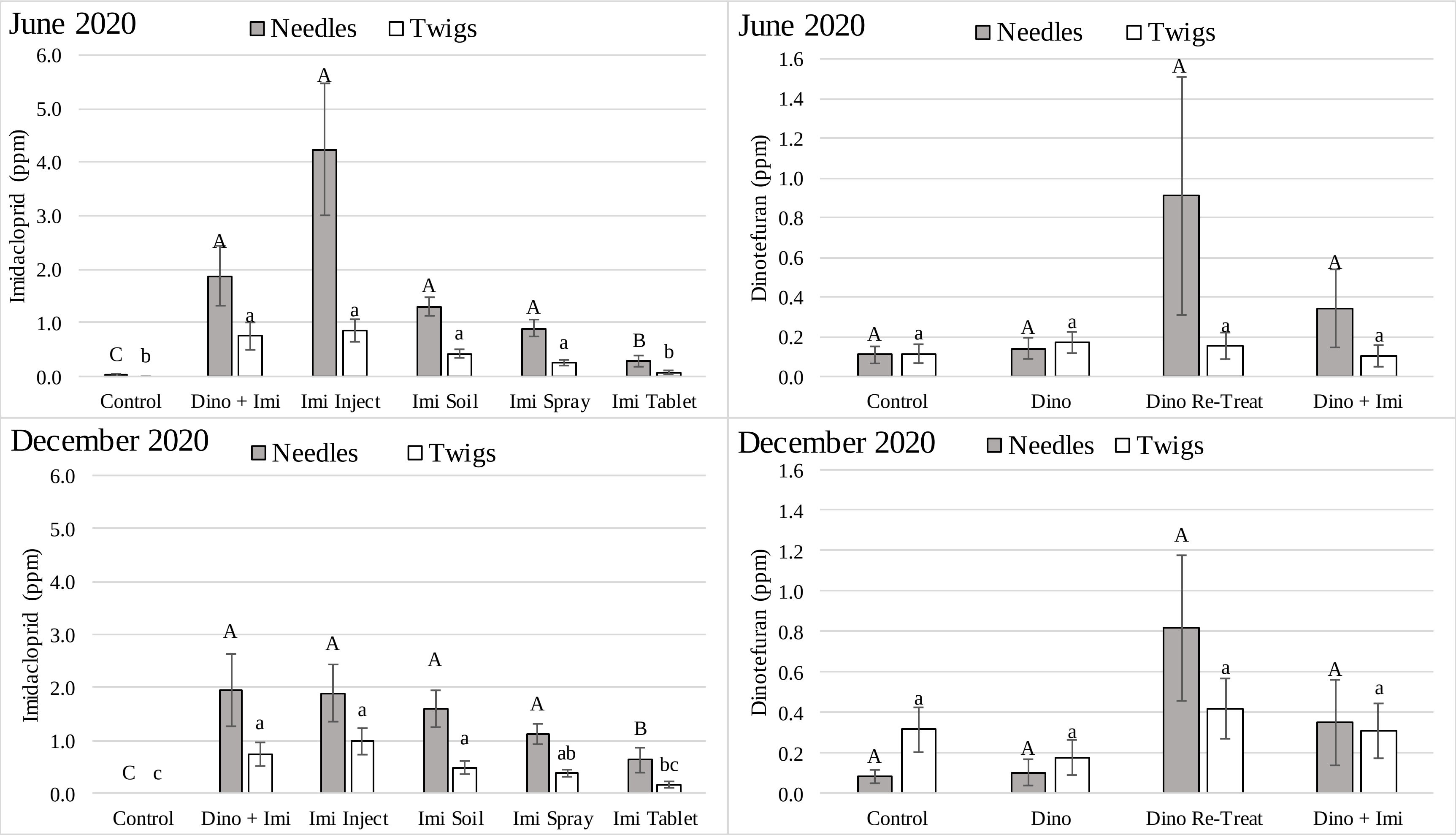

Figure 3.1. Mean (± SE) imidacloprid and dinotefuran residues quantified by commercial enzyme-linked immunosorbent assay (ELISA) kits in hemlock needles and twigs from trees treated in fall 2016 or spring 2017 (n = 10 trees per treatment) and sampled in June and December 2020. Capital letter codes above bars denote significant differences among treatments for needle samples, and lowercase letter codes for twig samples.

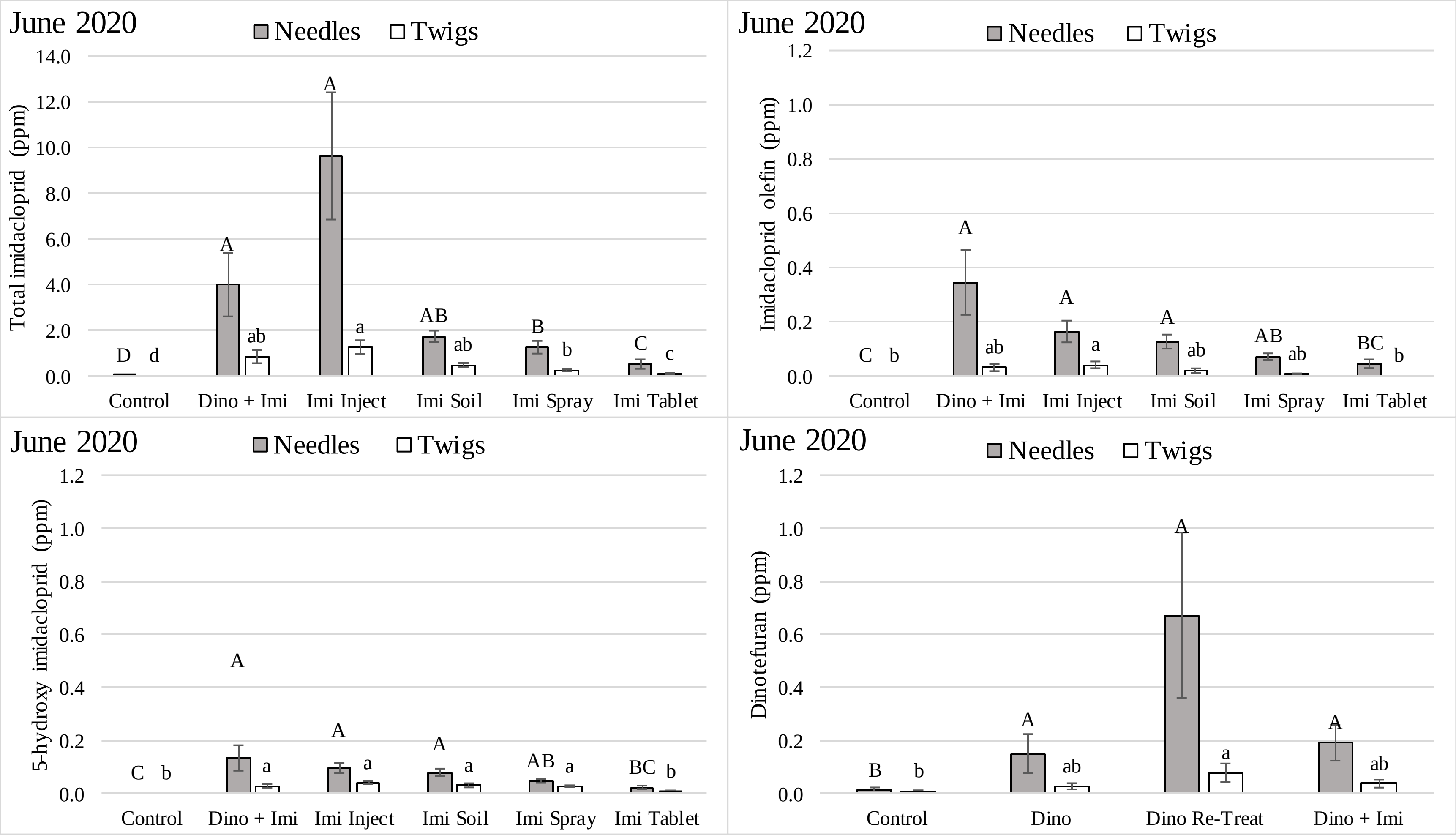

Figure 3.2. Mean (± SE) insecticide residues in needles and twigs from composite samples of hemlock shoots collected from Study 1 trees in June 2020 (n = 10 trees per treatment). Residues were quantified by ultra-high performance liquid chromatography with tandem mass spectrometry (UPLC/MS/MS) for total imidacloprid (including all metabolites), imidacloprid olefin, 5-hydroxy imidacloprid, and dinotefuran. Capital letter codes above bars denote significant differences among treatments for needle samples, and lowercase letter codes for twig samples.

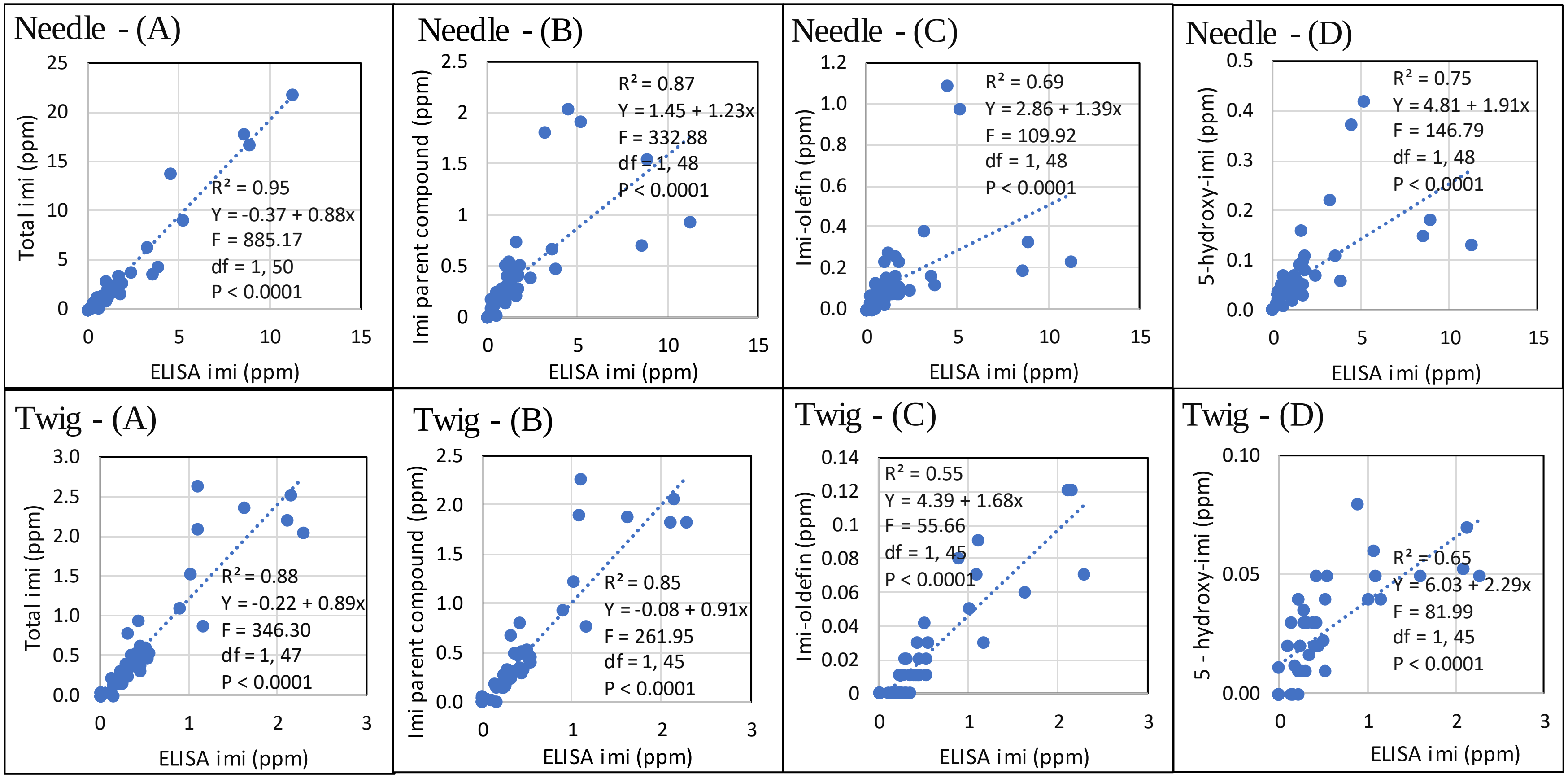

Figure 3.3. Linear relationships between imidacloprid residues (ppm) quantified by ELISA and LC/MS/MS in needles and twigs from composite shoot samples collected from Study 1 trees in June 2020 (n = 10 trees per treatment). Imidacloprid residues quantified by ELISA were compared to residues quantified by ultra-high performance liquid chromatography with tandem mass spectrometry (UPLC/MS/MS) for total imidacloprid including all metabolites (A), imidacloprid parent compound (B), imidacloprid-olefin (C) and 5-hydroxy imidacloprid (D).

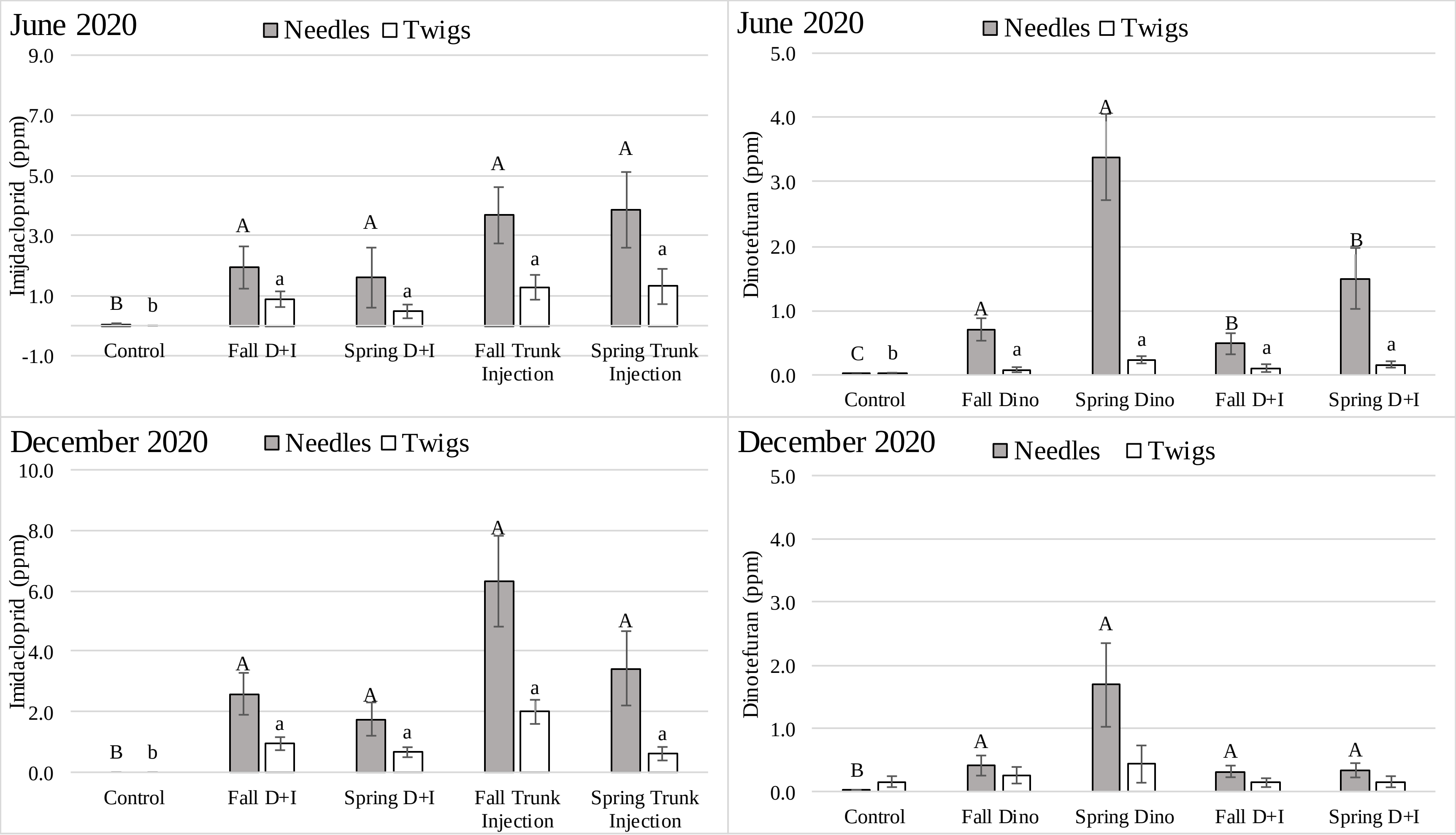

Figure 3.4. Mean (± SE) imidacloprid and dinotefuran residues quantified by commercial enzyme-linked immunosorbent assay (ELISA) kits in hemlock needles and twigs from composite shoot samples collected from Study 2 trees (n = 10 trees per treatment) in June and December 2020. Capital letter codes above bars denote significant differences among treatments for needle samples, and lowercase letter codes for twig samples.

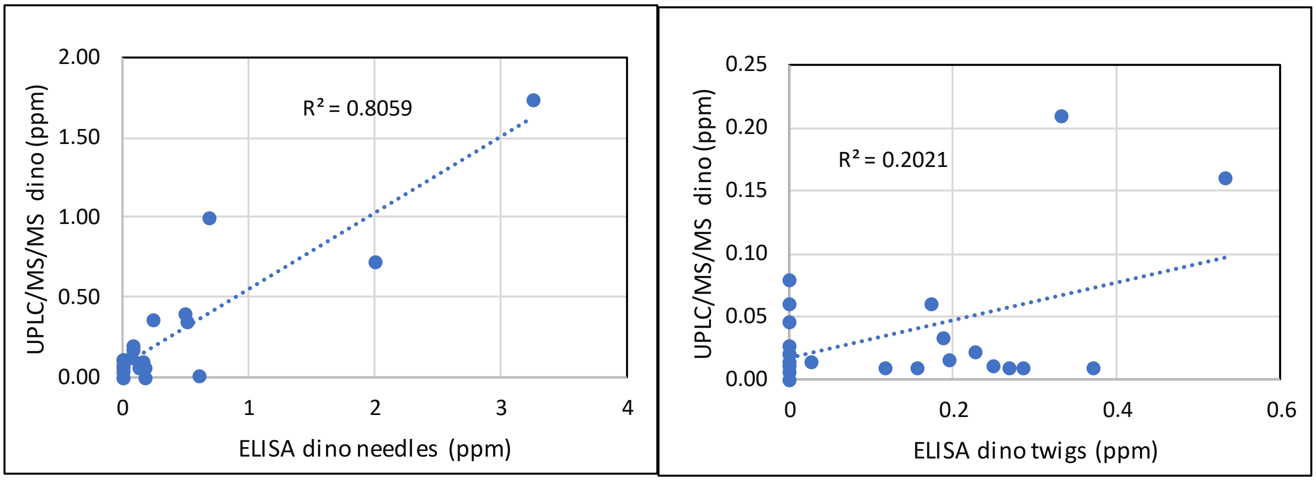

Figure 3.5. Linear relationships between dinotefuran residues (ppm) quantified by enzyme-linked immunosorbent assay (ELISA) and ultra-high performance liquid chromatography with tandem mass spectrometry (UPLC/MS/MS) residues in needles and twigs from composite shoot samples collected from Study 1 trees in June 2020. Trees were treated with basal trunk sprays of dinotefuran in 2016 (n = 10 trees per treatment) and re-treated in October 2018 (n = 5 trees).

CHAPTER 4: PERSISTENCE AND DISTRIBUTION OF DINOTEFURAN IN TREE OF HEAVEN

Introduction

Spotted lanternfly (SLF), *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), an invasive planthopper native to China and Taiwan, became established in Korea in 2004 (Kim et al. 2021) and was subsequently detected in the United States in Pennsylvania in 2014. Since then, established populations of SLF have been identified in localized areas of at least 14 states (New York State Integrated Pest Management 2022). Predictive models based on climate and host distribution suggest SLF could potentially become established across much of the eastern U.S (Jones et al. 2022, Wakie et al. 2019). Although SLF adults typically engage in migratory flights and disperse to nearby areas in late summer or fall (Wolfin et al. 2020), long distance spread occurs when people accidentally transport SLF life stages into new areas.

Research in field sites in Pennsylvania has confirmed the univoltine life cycle of SLF. Egg hatch begins in mid April and peaks in May (Dara et al. 2015, Liu and Hunter 2022). Nymphs feed throughout summer, completing four instars. Adults, which first appear in late July, feed intensively in aggregations during their four month life span (Liu 2019). Mating can occur from early September through late October and oviposition occurs from mid September to early November (Liu 2019, Liu and Hunter 2022). Each female lays 1-2 egg masses containing 30 to 50 eggs on tree trunks or branches or on hard, solid items including boulders, bricks, outdoor equipment and vehicles (Dara 2015, Liu 2019, 2020, Liu and Hunter 2022). Egg masses overwinter until hatching begins the following spring.

Adults and all nymphal stages feed on phloem sap, excreting copious amounts of honeydew, which leads to growth of black sooty mold (*Capnodium* spp. [Dothideales:

Capnodiaceae]) on host trees, vegetation and outdoor items below infested trees (Dara et al. 2015, Liu 2019). Black sooty mold reduces photosynthetic area of foliage, potentially affecting plant vigor as well as appearance, and contaminating agricultural crops (Barringer et al. 2015, Dara 2015, Liu 2019). Wasps and ants are often attracted to the sweet honeydew, causing further annoyance to residents in affected areas. Given the relatively long duration of SLF adult activity and the high densities SLF populations can reach, this insect can be a major nuisance for residents in affected areas. To date, SLF is not known to have caused tree mortality, although feeding has killed individual shoots or small branches of black walnut (*Juglans nigra* L. [Fagales: Juglandaceae]), maples (*Acer* spp. [Sapindales: Sapindaceae]) and other native trees. Intensive feeding combined with black sooty mold can also reduce yield, quality or simply render fruit from infested trees, grapevines (*Vitis riparia* Michx [Vitales: Vitaceae]) and hops (*Humulus* spp. [Rosales: Cannabinaceae]) unmarketable (Johnson et al. 2019, Liu 2019).

Although SLF can feed on several trees and woody vines, tree of heaven (ToH) (*Ailanthus altissima* (Mill.) [Sapindales: Simaroubaceae]) is the most preferred host for SLF feeding and reproduction (Liu 2019, Dara et al. 2015). ToH, native to China, was introduced into the U.S. in 1784 and was widely planted in urban areas through the 19th century (Miller 1990). It has subsequently spread across much of the U.S. Today, ToH is considered to be an undesirable invasive because of prolific seed production by female trees and high germination rates (Sladonja et al. 2015), its ability to colonize disturbed sites and outcompete more desirable vegetation, and the unpleasant odor of crushed leaves or twigs (Jackson et al. 2020). Tree of heaven can also reproduce clonally via sprouts from lateral roots (Kowarik 1995, O’Neal and Davis 2015, Sladonja et al. 2015) and may root graft with other ToH, monopolizing available nutrients in a site (O’Neal and Davis 2015). Because ToH is highly intolerant to shade (Knapp and Canham 2000, Miller 1990, Sladonja et al. 2015), it is rarely present in closed canopy forests but often grows along forest edges.

Early efforts to eradicate or contain SLF in Pennsylvania involved treating male ToH with dinotefuran, a systemic neonicotinoid insecticide commonly applied as a basal trunk spray (Urban et al. 2021). Although dinotefuran can be applied via trunk injection, basal trunk sprays are relatively efficient and can be used on trees that are small or otherwise difficult to treat with trunk injection. High rates of SLF mortality were consistently observed following dinotefuran treatment (Lewis 2018). At the same time, female ToH in areas with SLF infestations were removed or killed with herbicide (Urban et al. 2021). This encouraged SLF to feed on the treated trap trees and also limited further ToH reproduction.

While SLF eradication is no longer a realistic objective, dinotefuran continues to be widely used for control of SLF in Pennsylvania and more recently infested states (Leach et al. 2021). Because dinotefuran is highly water-soluble, it is translocated relatively rapidly in trees compared to imidacloprid, another systemic neonicotinoid insecticide, but is less persistent (Cowles 2006, Doccola et al. 2007, Herms et al 2019, McCullough et al. 2007, 2011). For example, in ash (*Fraxinus* spp. [Lamiales: Oleaceae]) trees treated in May, foliar imidacloprid residues continued to increase through the growing season while dinotefuran levels were dropping by late summer (McCullough et al. 2007, McCullough et al. 2011, Mota-Sanchez et al. 2009, Tanis et al. 2015). Recent studies have shown other insecticides, including cover sprays of broad spectrum pyrethroid products, can effectively control SLF nymphs or adults (Leach et al. 2019, Lee et al. 2019). However, given concerns about insecticide drift, impacts on nontarget insects and the difficulty of effectively spraying tall trees, dinotefuran remains an essential tool for SLF management.

Identifying the optimal timing for basal trunk sprays of dinotefuran is an essential aspect of SLF containment and management programs, given that feeding extends for at least six months, and label restrictions prohibit multiple applications in a single year. In a previous study, dinotefuran residues in foliage sampled from ToH treated in May persisted into September, whereas in trees treated in April, residues sharply declined between August and September (Lewis 2020). Spring applications of dinotefuran reduce early instar densities, protecting trees and vines from feeding, honeydew and sooty mold growth. Whether insecticide residues remain adequate to control fourth instars and adults in late summer or autumn when feeding and honeydew production are most intense, however, remains a key question for pest managers. Additionally, when SLF nymphs are not controlled, mature adult females commonly engage in short-range dispersal flights (Wolfin et al. 2019, 2020), sometimes invading vineyards and orchards where late season insecticide sprays just before or during harvest, are especially problematic. Because resources are rarely sufficient for multiple insecticide applications in a single year, understanding translocation and persistence of dinotefuran can help pest managers efficiently control SLF densities while limiting unnecessary applications.

Systemic insecticides such as dinotefuran are transported in xylem tissue (Mota-Sanchez et al. 2009, Tanis et al. 2012) and accumulate in leaves, which function as a major sink for water and nutrients during much of the growing season. Insecticide residues in foliage samples are frequently used to quantify insecticide concentrations, monitor insecticide persistence over time or to compare treatment timing, application methods or other factors. All SLF nymphal stages and adults, however, feed on phloem in tree branches and trunks (Dara et al. 2015, Leach and Leach 2020, Liu 2019). Observations of high and often rapid SLF mortality following dinotefuran application (Leach et al. 2019, Leach 2020, Lewis 2018, 2020) suggest that either dinotefuran moves into the phloem, i.e., via transverse rays, or the mouthparts of SLF insects penetrate phloem and encounter insecticide in xylem vessels. Evaluating dinotefuran presence and concentrations in phloem could help to fully understand options for optimizing SLF control.

We conducted two studies in 2019 to assess dinotefuran persistence and within-tree distribution following basal trunk sprays applied to healthy ToH in sites in Michigan, well beyond any known SLF infestation. In the first study, dinotefuran was applied in early April and residues were quantified in samples of ToH foliage collected periodically until late September when leaves were dropping. Based on previous research and experience, we expected dinotefuran residues would remain relatively high for at least two months before declining in mid to late summer. We also evaluated whether tree diameter affected foliar dinotefuran concentrations at each sampling period. We expected to find little or no relationship between residue levels and tree diameter, given that label application rates are based on tree DBH (diameter at breast height) and the thin outer bark of ToH seemed unlikely to prevent rapid movement of dinotefuran into xylem tissue.

In the second study, we quantified residues in ToH foliage and phloem collected on two post-treatment dates following basal trunk sprays of dinotefuran applied in June. Foliage and phloem samples were collected from trees felled in either July or September to compare dinotefuran levels in the two tissues and to assess potential effects of aspect, sampling dates and tree DBH on dinotefuran concentration. Phloem samples were collected from below and above the spray line on trunks of trees felled in July and trees felled in September. We expected phloem residues below the spray line to decrease over time as insecticide was transported to the canopy but whether dinotefuran would be detectable above the spray line, especially in September, was unknown. Given the many reports of rapid mortality of SLF nymphs and adults on trees treated with dinotefuran (Leach et al. 2021, Lewis 2018, 2020, Urban et al. 2021), we anticipated that dinotefuran would be present in phloem from branches, although perhaps at lower levels than in foliage. We also were interested in determining whether the relative sun exposure of leaves and branches affected dinotefuran levels or persistence.

Materials and Methods

Study Sites: Study 1 was conducted with ToH growing in an unmanaged, ~0.1 ha strip of land in Lansing, Ingham County, Michigan. The site was in an industrial area with an overstory composed entirely of ToH, and an herbaceous layer of poison ivy (*Toxicodendron radicans* (L.) Kuntze [Sapindales: Anacardiaceae]), and Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch [Vitales: Vitaceae]). On 2 April 2019, 24 ToH trees with DBH ranging from 10.9 to 34.8 cm DBH and averaging 19.3 ± 1.4 cm were selected and tagged. Twenty trees were assigned to a basal trunk spray of dinotefuran and four were left as untreated controls. Brush was cleared around each tree to facilitate access.

Study 2 was conducted in an ~0.4 ha, even-aged plantation of ToH established in 1976 at MSU's W.K Kellogg Forest in Augusta, Kalamazoo County, MI. A few northern red oak, *Quercus rubra* (L.) [Fagales: Fagaceae] trees grew along the plantation borders while black cherry (*Prunus serotina* (Ehrh.) [Rosales: Rosaceae]) saplings and European buckthorn, (*Rhamnus cathartica* (L.) [Rosales: Rhamnaceae]) grew between and within the rows of ToH. Herbaceous vegetation was dominated by poison ivy, multiflora rose (*Rosa multiflora* (Thunb.) [Rosales: Rosaceae]) and wild raspberry (*Rubus* sp. [Rosales: Rosaceae]) shrubs. On 16 May 2019, brush was cleared at the site (using hand tools) to facilitate access to the trees and to allow a skidsteer to maneuver between and within rows. We tagged and measured DBH of 26 trees across the plantation. Tree DBH ranged from 7.1 to 37.6 cm DBH and averaged 18.8 ± 1.2 cm.

Six trees were randomly selected to be left as untreated controls while the remaining 20 were treated with a basal trunk spray of dinotefuran. Even-numbered treated and control trees were felled in mid-summer while odd-numbered treated and control trees were felled in late summer (see below).

Cumulative growing degree days corresponding to each treatment and sampling date were acquired from data recorded by MSU EnviroWeather stations located at the MSU Horticulture Teaching and Research Center, approximately 13 km from the Study 1 site, and from the MSU Kellogg Biological Station, approximately 8 km from the Study 2 site. Cumulative growing degree days were calculated using the Baskerville-Emin method with a base 10°C developmental threshold and a starting date of 1 January. Growing degree day accumulations corresponding to treatment and sampling dates are reported here for potential application in other regions with different weather regimes.

Dinotefuran Application: Trees in Study 1 and Study 2 were treated with dinotefuran on 9 April (25 GDD [growing degree days]) and 6 June (291 GDD) 2019, respectively, using the same insecticide rate and application method. Twelve water soluble packets of Transtect® were added to 3.8 liters (one gallon) of distilled water in the tank of a low-pressure 7.5 liter garden sprayer. Formulated insecticide was applied as a basal trunk spray at a rate of 23 ml (0.78 oz) per cm DBH (0.6 g active ingredient per cm DBH) to tree trunks from approximately 1.5 m high down to the base, ensuring the entire trunk was covered and the appropriate amount of insecticide was applied. Spray was applied at low pressure to minimize any drift around tree trunks and care was taken to avoid any spray contact with designated control trees.

Sampling: To account for the often irregular crown shape of ToH (Knapp and Canham 2000), composite foliage samples from Study 1 trees were comprised of shoots from branches on at least three different aspects, whenever available. Leaf-bearing shoots were clipped from treated and control trees on 25 July (770 GDD),107 days post-treatment. Foliage samples from each tree were placed into labeled bags, returned to the MSU Forest Entomology Laboratory in coolers with blue ice, then frozen. In the lab, leaflets were stripped from petioles, and petioles and woody twigs were discarded. Sampling was repeated on 20 Aug (1077 GDD) and 30 Sept (1425 GDD), at 133 days and 174 days post-treatment, respectively.

For Study 2, half of the trees in the plantation were destructively sampled on 16 July 2019 (771 GDD), 40 days post-treatment. The spray line on each tree trunk was marked, then a skidsteer felled ten of the treated trees and three untreated control trees. Trees were cut at approximately the top of the spray line.

Leaves were collected with hand pruners from canopy branches on three to four aspects of the felled trees, depending on crown structure, and bagged separately by aspect for each tree. Phloem samples were collected from the same canopy branches using drawknives to remove long strips of bark and phloem beginning near the trunk and extending distally until the branch was ≤ 4-5 cm in diameter. Heavy overcast conditions, however, limited our ability to confidently assess relative amounts of sun or shade exposure of individual branches. Drawknives were also used to remove 0.5 to 1.0 m long strips of bark and phloem from the upper half of the trunk on the felled trees. Samples from above the spray line were collected 2.5 to 4 m above ground and samples from below the spray line were collected 0.5 to 1.0 m above ground, within the area that had been sprayed. Phloem readily separated from xylem and outer bark in the branch and trunk samples. Phloem samples from different branches and from above and below the spray line on tree trunks were placed into individual bags. All drawknives and hand pruners used for sampling were sterilized with 70% ethanol between each sample to avoid contamination.

Remaining trees in Study 2 in the plantation were felled and sampled on 17 September (1478 GDD), 103 days post-treatment, using the same methods as above. Exposure to sun, which could presumably affect insecticide concentration or persistence, was qualitatively ranked for each branch that was sampled as 1 if it was fully shaded, 2 if it was partially shaded and 3 if it was fully exposed to sunlight.

Foliage and phloem samples collected in July or in September from the Study 2 trees were bagged, transported in a cooler with blue ice to the MSU Forest Entomology Laboratory, then frozen as in Study 1. Leaflets were stripped from shoots and petioles in the laboratory, then re-frozen. Frozen foliage and phloem samples were shipped overnight to collaborators at the USDA APHIS laboratory in Buzzards Bay, MA on 22 October 2019 for insecticide residue analysis.

Residue analysis: Foliage and phloem samples were removed from bags, air-dried for at least two weeks, then ground in a commercial blender to a fine powder. Blenders were tripled rinsed, scrubbed with soapy water (LIQUINOX® detergent, Alconox Inc., White Plains, NY) and a bottle brush, sprayed with 95% ethanol then rinsed in dionized water to ensure any insecticide residue was removed. Personnel changed nitrile gloves between samples to further minimize any risk of cross contamination.

Analysis of insecticide residues in ToH leaves collected from trees in Study 1 and Study 2, and in ToH phloem from Study 2 trees was determined using commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits (FujiFilm/Horiba; Kyoto, Japan and Wako Chemical, USA Corporation, Richmond, VA). A 0.5 g sample of processed plant material was weighed into a 50 mL plastic centrifuge tube and extracted in 10 mL of pure methanol for 3 hrs on a table-top shaker. Sample tubes were spun down in a high-speed centrifuge for 10 min and the supernatant diluted a minimum of 20x to avoid matrix effects from the kit due to the methanol. Sample aliquots were added to a 96-well plate, developed and the absorbance value calculated according to the manufacturer's instructions using provided standards of 1.5 ppb to 30 ppb. The effective lower limit of kit detection following sample preparation and dilution is 0.6 ppm.

Statistical Analysis: Normality of dinotefuran residues in foliage from Study 1 trees was assessed with a Shapiro-Wilk test and residual plots (PROC MIXED, PROC UNIVARIATE, SAS 9.4) and a square root transformation was applied to normalize residue data (Pr <W = 0.1456).

A one-way ANOVA with repeated measures (PROC MIXED, SAS 9.4) was used to compare differences in foliar dinotefuran residues among the three sample dates with an *a priori* significance level of $\alpha = 0.05$. The Kenward-Roger correction was used for calculating denominator degrees of freedom because it is more conservative than the MIXED default and is generally recommended for repeated measures analysis to minimize the risk of an increased Type 1 error rate generated by improperly fitted covariance structure. The Tukey-Kramer multiple comparison test was applied when the ANOVA results were significant to identify significant differences among sampling dates. Additionally, linear relationships between foliar dinotefuran residues and tree diameter were assessed with simple linear regression (PROC REG).

Results of a Shapiro-Wilk test and residual plots (PROC UNIVARIATE, SAS 9.4) showed dinotefuran residues in leaf samples collected from the Study 2 trees in the plantation were not normally distributed and data were not normalized by transformation. A two-way nonparametric ANOVA was therefore performed on ranked foliar insecticide residues to assess differences among leaves collected from branches on different aspects of the canopy and between the two sample dates (PROC RANK, PROC MIXED, SAS 9.4). Dinotefuran was not detected in any of the phloem samples collected from branches on either sampling date.

A composite foliar residue value for each tree in Study 2 was calculated by averaging residues in the leaves from the three to four sampled branches in July and again in September. Composite foliar residue values in trees were normal on both sampling dates. An independent t-test was used to assess differences in foliar residues between samples collected from trees felled in July versus September (PROC TTEST, SAS 9.4). Within each month, differences between foliar and trunk phloem residues and between residues in phloem from above and below the spray line were evaluated with paired t-tests. Simple linear regression (PROC REG, PROC UNIVARIATE, SAS 9.4) was applied to assess relationships between foliar residue levels and tree DBH for trees sampled on each date.

Results

Study 1: As expected, foliar dinotefuran residues from Study 1 trees were significantly higher in foliage from treated trees than in untreated controls, which had no dinotefuran, across all months (F = 20.52; df = 1,21.9; $P < 0.001$) and differed among post-treatment sample dates (F = 5.63; df = 2,34.6; $P = 0.0076$) (Figure 4.1). Residues averaged 7.8 ± 1.1 ppm and ranged from 0.7 to 17.0 ppm in July, 6.3 ± 1.2 ppm and 0 to 20.0 ppm in August, and 2.6 ± 0.5 ppm and 0 to 8.8 ppm in September. Residues were significantly higher in samples collected in July (770 GDD) than in September (1425 GDD) ($P < 0.001$) and in August (1077 GDD) compared to September ($P = 0.0013$), but the drop in average dinotefuran concentration between July and August was not significant ($P = 0.5412$) (Figure 4.1). Residues in 14 trees were lower in August than in July, while values increased in the six trees between July and August. Between August and September, residues in 18 of the 20 treated trees had declined and overall residues in September were 50% lower than in August and 66% lower than in July. Residues in two trees increased slightly in September from August, but residue values were substantially lower in these trees from the July values. Tree size did not affect foliar residues in any of the sampling periods; simple linear regressions yielded R^2 values of 0.02 ($P = 0.48$), 0.03 ($P = 0.41$) and 0.002 ($P = 0.85$) in July, August and September, respectively.

Study 2: Dinotefuran residues were detected in foliage from all treated trees sampled in mid-July (771 GDD), 40 days post-treatment, and in mid-September (1478 GDD), 103 days post-treatment. Mean foliar residue levels averaged 12.7 ± 1.32 and 14.6 ± 2.18 ppm in the ten trees felled and sampled in July and the other ten trees sampled in September, respectively. While average foliar residues were approximately 5% higher in September than in July, the difference was not significant (t = -0.55; df = 1,23; $P = 0.5895$). Results from the two-way ANOVA confirmed the similarity in foliar residues between samples collected in July and September (F = 0.40; df = 1,87; $P = 0.53$). Residues also did not differ among foliage samples collected from branches at different aspects (F = 0.02; df = 3,87; $P = 0.89$). Mean foliar residues ranged from 11.9 ± 1.90 ppm (eastern aspect) to 13.5 ± 2.69 ppm (southern aspect) in July and from 12.4 ± 3.78 ppm (southern aspect) to 16.3 ± 5.50 ppm (northern aspect) in September. As in Study 1, tree DBH did not affect mean foliar residues in trees sampled in either July ($R^2 = 0.16$; $P = 0.25$) or September ($R^2 = 0.0002$; $P = 0.97$). Leaves from one tree sampled in September exhibited an unusually high dinotefuran concentration but excluding this outlier had little effect on results ($R^2 = 0.03$; $P = 0.621$). All branches that were sampled to collect foliage and phloem from trees felled in September were either partially (Rank 2) or fully exposed to sun (Rank 3). There was no evidence that sun exposure affected foliar dinotefuran residues.

Dinotefuran residues in phloem samples collected from above and below the spray line on trunks of the felled trees varied substantially and were often too low to be detected. Phloem samples collected in July from below the spray line yielded detectable dinotefuran residues in seven of the ten felled trees, averaging 8.6 ± 4.4 and ranging from 0.8 to 32.2 ppm. Three trees had detectable levels of dinotefuran in phloem from above the spray line, with concentrations ranging from 1.6 to 10.6 ppm and averaging 7.4 ± 2.9 ppm. Phloem from only one tree had detectable dinotefuran residues in samples from both above (9.8 ppm) and below the spray line (0.8 ppm).

In September, none of the phloem samples collected from above the spray line on the trunks of the ten felled trees had detectable dinotefuran residues. Seven of these trees had measurable dinotefuran residues in phloem from below the spray line, ranging from 0.8 to 10.8 and averaging 3.7 ± 1.3 ppm.

Overall, residues in phloem samples collected from tree trunks were significantly lower than residues in foliage collected from the same trees in July (t = 3.60; df = 9; *P* = 0.0058) and September (t = 4.31; df = 9; *P* = 0.002). On average, phloem residues in samples from below the spray line were 53% lower than foliar residues in July and 83% lower than September foliar residues. Phloem residues in samples from above and below the spray line did not significantly differ in July (t = 0.99; df = 9; *P* = 0.35) but were significantly higher below the spray line than above the spray line in September (t = 2.49; df = 9; *P* = 0.03). None of the phloem samples collected from branches had detectable dinotefuran residues, regardless of sample date or aspect.

Discussion

Basal trunk sprays of dinotefuran remain a key tool for managing SLF infestations to reduce insect density, protect the health of trees and other hosts, and lessen the annoyance or anxiety experienced by residents during outbreaks. Identifying the optimal time to apply dinotefuran, however, remains an essential question for pest managers dealing with established SLF populations along with newly discovered infestations. Regulatory personnel, IPM specialists and resource managers desire a high level of SLF control that is also cost-effective and logistically practical (Leach et al. 2021). Launching dinotefuran applications in spring could be advantageous when extensive areas require treatment, especially if personnel or funding are likely to be limited later in the season. Reducing densities of SLF early instars in an area also decreases feeding and honeydew production by later life stages, minimizing potential injury to host plants. Conversely, in other situations, SLF infestations may not be discovered until late summer or autumn when brightly colored 4th instars or the large adults are more easily observed. High densities of SLF can also appear in previously uninfested areas following migratory flights by mature adults in late summer or fall (Wolfin et al. 2020).

In our studies, as in most research with systemic insecticides, residues in samples of foliage were quantified to evaluate persistence of dinotefuran. Sampling leaves to assess insecticide concentrations causes minimal injury to trees and facilitates repeated sampling over time. Tree DBH, which ranged from 10.9 and 7.1 cm up to 34.8 and 37.6 in Study 1 and Study 2, respectively, did not affect foliar dinotefuran residues in any sampling period. This is not surprising since the amount of insecticide applied to any tree is based on the DBH of the tree. Previous studies with ash trees, which are ring porous like ToH, have shown that systemic insecticides are carried in xylem vessels in the outer ring of sapwood from the trunk to the canopy, where expanding buds and leaves act as a strong sink for xylem (Mota-Sanchez et al. 2009, Tanis et al. 2012, McCullough et al. 2019). It is notable, however, that the thicker outer bark on large trees relative to the smaller trees in this study did not limit absorption nor affect translocation of dinotefuran applied via basal trunk sprays in early April (Study 1) or June (Study 2). Age of the largest trees in Study 1 are unknown, but records show that the mature trees in the plantation used for Study 2 were 48 years old at the time of treatment and sampling. A high proportion of trees in most areas where SLF is established will likely be of similar size and can be efficiently treated with basal trunk sprays instead of more laborious trunk injections. Since ToH trees can reportedly attain diameters of >1.5 m (Jackson et al. 2020), however, further evaluation of insecticide translocation in very large trees may be warranted.

While foliar residues are ideal for monitoring insecticide presence over time or comparing different treatments, SLF feeds by sucking phloem sap from tree branches, trunks, and woody vines. Several studies have reported high and relatively rapid SLF mortality following dinotefuran application (Leach et al. 2019a, 2019b, Leach 2020, Lewis 2018, 2020), indicating that these insects must encounter lethal levels of insecticide as they feed. We anticipated dinotefuran residues would be relatively high in phloem samples collected from below the spray line on tree trunks, e.g., the area where the dinotefuran spray was physically applied. We also expected to detect some level of dinotefuran in phloem samples from above the spray line and in branches, indicative of dinotefuran translocation to the canopy. Movement of dinotefuran from xylem into phloem via transverse rays could presumably result in the consistently high SLF mortality observed on treated trees (Lewis 2018, 2020). However, in the ten Study 2 trees sampled in July, only 40 days post-treatment, dinotefuran was undetectable below the spray line in three trees and above the spray line in seven of the trees. The lack of detectable dinotefuran residues in phloem from any of the branches sampled on the Study 2 trees was also unexpected, particularly given the insecticide levels in leaves from those same branches. It is possible that dinotefuran in the phloem samples from the branches was present at concentrations below the detection limit of 0.6 ppm of our assay. While LC_{50} values for dinotefuran corresponding to SLF mortality are unknown, it seems unlikely that residues consistently below detection limits would cause the high SLF mortality rates previously observed in multiple infestations (Lewis 2018, 2020).

A possible mechanism to account for these seemingly contradictory observations is that while SLF need to access nutrients in phloem, their mouthparts may penetrate phloem and reach xylem tissue in the outer sapwood ring, which could result in the insects encountering a lethal dose of insecticide. Research has suggested that phloem-feeding emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire [Coleoptera: Buprestidae]) larvae may similarly encounter insecticide when early instar galleries score the outer xylem in ash trees (McCullough et al. 2011, 2019). Further research into the mechanics of SLF feeding is needed to understand how these insects encounter systemic insecticides, particularly small early instars with short stylets (Avanesyan et al. 2019).

When young ash (*Fraxinus* spp.) trees were injected with ^{14}C-labelled imidacloprid, another systemic neonicotinoid, residues in subsequent foliage samples varied depending on the position of branches relative to injection sites, and with the height of branch whorls (Tanis et al. 2012). Translocation patterns of ToH and ash, both ring porous trees, are probably similar but in our Study 2 trees, foliar residues were not affected by aspect of the leaf-bearing branches we sampled. Basal trunk sprays, which are applied around the entire circumference of the tree, may facilitate a more even distribution of insecticide throughout the canopy than trunk injection. Additionally, we hypothesized that higher transpiration rates in leaves fully exposed to sun could result in more rapid translocation or higher residues, at least initially, than in shaded branches. However, we found no evidence that exposure to sunlight affected insecticide translocation rates or persistence in foliage. Virtually all foliage-bearing branches on trees in both Study 1 and Study 2 were at least partially exposed to sunlight, while branches below the canopy or those that were shaded by adjacent trees were dead, a pattern consistent with the low shade tolerance exhibited by ToH, and its rarity in closed canopy forests (Knapp and Canham 2000, Swearingen and Pannill 2009).

Although dinotefuran LC_{50} values for SLF have not been determined, we assumed that trees with high dinotefuran concentrations in leaves would be more toxic to SLF nymphs and adults than trees with lower residues. Foliar residues from Study 2 trees, treated in June (291 GDD), averaged 12.8 ± 1.3 and 14.6 ± 2.2 at 40 and 103 days post-treatment, respectively, while residues in Study 1 trees, treated in early April (25 GDD), averaged 7.8 ± 1.1 and 6.3 ± 1.2 ppm in samples collected in July and August, 107 and 133 days post-treatment, respectively. Generally lower foliar residues in Study 1 trees compared with Study 2 trees may reflect the poor Study 1 site conditions, reflected in lower respiration and translocation rates. Study 1 trees were in a narrow, highly disturbed strip of land bordered by parking lots, while Study 2 trees were on a relatively high quality site with minimal disturbance. Variability in foliar residues among Study 1 trees, as evidenced by standard errors, increased between July and August (albeit slightly), and between July and Sept for Study 2 trees, a pattern consistent with differences among trees in insecticide translocation. Increased foliar residues in Study 2 trees between 40 and 103 days post-treatment presumably reflects continued translocation of insecticide from the lower trunk to canopy branches and leaves. Six of 20 Study 1 trees had higher residues in mid August (133 days post-treatment; 1077 GDD) than in late July (771 GDD), indicating translocation of insecticide was still occurring between 107 and 133 days post-treatment in some trees.

Collectively, these results suggest basal trunk sprays should provide effective control of SLF for at least 100 days and probably for as much as 135 days post-treatment in most trees. However, residues dropped sharply in Study 1 trees during the 41 days between the mid August and late September samples, when residues averaged < 3 ppm (174 days post-treatment). Similarly, the number of Study 1 trees with relatively low foliar residues, e.g., ≤ 5 ppm, increased from seven trees in the July samples, to 12 trees in August and 18 trees in September. In an earlier study, dinotefuran residues in trees treated in mid to late May remained relatively stable in September (Lewis 2020).

Early season treatments to reduce densities of early instars would presumably limit feeding, honeydew production and associated impacts in a given area throughout the summer. However, applications made too early will likely result in trees with relatively low and rapidly declining residues from late August through October, a period when SLF adult feeding, dispersal and migratory flights are likely to peak (Baker et al. 2019). Delaying dinotefuran basal trunk sprays until late May or mid June should provide effective control of late instars and SLF adults in October, although early instar feeding and local dispersal would still occur. Understanding more about translocation and persistence of dinotefuran and other systemic insecticides including imidacloprid would be valuable for SLF programs and more broadly for insect pests of other trees.

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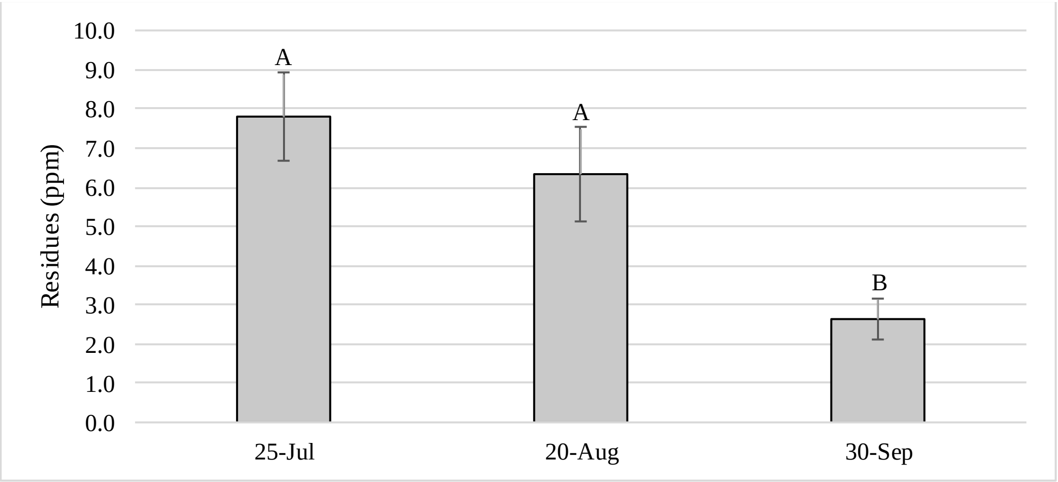

Figure 4.3. Mean (±SE) dinotefuran residues (ppm) in foliage samples from *Ailanthus altissima* trees in Study 1. Samples were collected periodically in 2019 following a 9 April 2019 basal trunk spray. Letters above bars indicate significant differences (*P*<0.05) (n=20 trees).