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Uptake and Dissipation of Neonicotinoid Residues in Nectar and Foliage of Systemically Treated Woody Landscape Plants

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Abstract: Systemic neonicotinoid insecticides used in urban arboriculture could pose a risk to bees and other pollinators foraging on treated plants. We measured uptake and dissipation of soil-applied imidacloprid and dinotefuran in nectar and leaves of 2 woody plant species, a broadleaf evergreen tree (*Ilex* × *attenuata*) and a deciduous shrub (*Clethra alnifolia*), to assess concentrations to which pollinators and pests might be exposed in landscape settings. Three application timings, autumn (postbloom), spring (prebloom), and summer (early postbloom), were evaluated to see if taking advantage of differences in the neonicotinoids' systemic mobility and persistence might enable pest control while minimizing transference into nectar. Nectar and tissue samples were collected from in-ground plants and analyzed for residues by high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) in 2 successive years. Concentrations found in nectar following autumn or spring applications ranged from 166 to 515 ng/g for imidacloprid and from 70 to 1235 ng/g for dinotefuran, depending on plant and timing. These residues exceed concentrations shown to adversely affect individual- and colony-level traits of bees. Summer application mitigated concentrations of imidacloprid (8–31 ng/g), but not dinotefuran (235–1191 ng/g), in nectar. Our data suggest that dinotefuran may be more persistent than is generally believed. Implications for integrated pest and pollinator management in urban landscapes are discussed. *Environ Toxicol Chem* 2017;9999:1–11. © 2017 SETAC

Keywords: Imidacloprid; Dinotefuran; Neonicotinoid; Nectar; Bee; Urban landscape; Insecticide; Pesticide risk assessment; Invertebrate toxicology

INTRODUCTION

Neonicotinoid insecticides, particularly the nitroguanidine compounds imidacloprid and dinotefuran, are widely used in urban arboriculture for managing insect pests of trees and shrubs. Their targets include invasive species such as emerald ash borer (*Agrilus planipennis* Fairmare), Asian long-horned beetle (*Anoplophora glabripennis* Motschulsky), hemlock woolly adelgid (*Adelges tsugae*), and Japanese beetle (*Popillia japonica*) as well as aphids, psyllids, scale insects, leaf miners, and other pests [1–7]. Neonicotinoid insecticides can be applied via foliar sprays, but they are more frequently used as systemic treatments via trunk injection or infusion, basal bark sprays, or soil injections or drenches. Systemic application is increasingly preferred for treating woody landscape plants because it can be done without specialized equipment and minimizes spray drift, applicator and bystander exposure, visual anxiety associated with spraying in public places, and direct exposure of beneficial insects and other nontarget organisms [8,9]. When neonicotinoids are applied to the soil, they are taken up by the roots and move acropetally in xylem sap [10], enabling them to reach pest feeding sites (e.g., phloem–xylem interface under bark, upper-canopy foliage of trees) that are impossible or impractical to protect with nonsystemic spray applications.

Neonicotinoids may also be translocated into nectar or pollen, the principal food sources for bees [10]. Both imidacloprid and dinotefuran are acutely toxic to bees [11]. Furthermore, imidacloprid breaks down into additional insecticidal metabolites, including imidacloprid olefin and 5-hydroxy-imidacloprid, the former of which can be more acutely toxic to honeybees than imidacloprid itself [12,13]. Potential risk to bees is a major reason that neonicotinoids are under regulatory scrutiny [14,15]. Neonicotinoids are currently facing pressure from environmental advocacy groups calling for restrictions on their use [16].

Globally, bee populations face pressures from habitat loss and fragmentation, exotic pests and pathogens, nutritional stress, pesticide exposures, loss of genetic diversity, and other stressors [17,18]. In urban areas, bees provide pollination

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services to community and residential gardens, to ornamental fruit-bearing trees and shrubs that feed birds and other desirable wildlife, and to many wild plant species [19–25]. Urban bee communities are dominated by polylectic species [19,20,26–28] that collect nectar and pollen from diverse plants, including flowering trees and shrubs [29–31], so it is important that those floral resources not contain harmful levels of pesticide residues.

Many laboratory and semifield studies have demonstrated that exposure to food spiked with sublethal concentrations of neonicotinoids can adversely affect bees' learning, foraging ability, homing ability, and reproduction [32–34]. Less clear, however, is whether bees' exposure to neonicotinoids as they are normally used in the field is sufficient to cause such effects [32,35–37].

Presently there are almost no published data concerning rates of uptake and dissipation of neonicotinoid residues in nectar or pollen of woody landscape plants. Such information is needed to assess the range of concentrations to which bees might be exposed in urban landscape settings. Soil-applied imidacloprid provides multiple years of protection from some foliage–feeding pests of trees and shrubs [4,5,38,39], indicating the relative stability of it and its metabolites once absorbed by the plant. Dinotefuran is more water-soluble than imidacloprid, 39.8 vs 0.61 g/L, respectively [10], and has lower sorption to soil organic matter. Therefore, it tends to show more rapid uptake and translocation but also more rapid decline in plant tissues [40–43]. Manipulating application timing and taking advantage of the differences in systemic mobility and residual activity of imidacloprid and dinotefuran may be a way to minimize hazard to bees and still enable effective pest control for flowering woody landscape plants.

Our objectives for the present study were to measure uptake and dissipation of residues of soil–applied imidacloprid and dinotefuran in nectar and foliage of 2 species of established woody landscape plants using autumn (postbloom), spring (prebloom), and summer (postbloom) timings. The end goal is to support protocols for integrating pest and pollinator management for urban landscapes.

MATERIALS AND METHODS

Plant species and study sites

Two species of woody landscape plants served as models in independent trials: Foster holly (*Ilex* × *attenuata*; Aquifoliaceae), a dioecious broadleaf evergreen tree that is a natural hybrid of *Ilex opaca* (American holly) and *Ilex cassine*, both of which share native territory in the southeastern United States [44], and summersweet, also called sweet pepperbush (*Clethra alnifolia*; Clethraceae), a small native deciduous shrub. At the latitude of Kentucky, USA, Foster holly (*Ilex*) produces greenish white flowers (6–7 mm diameter, 3 mm deep) and typically blooms in mid-May to mid-June. Summersweet (*Clethra*) produces white flowers (8 mm diameter, 7 mm deep) in upright spikes and blooms in late July to early August. Both species are highly attractive to bees [31] and representative of large groups of common woody landscape plants (small, deciduous shrubs and evergreen trees).

The *Ilex* (~ 2.13 m tall; planted in 2009) were in an established hedge (180 same-age, uniform-sized trees) on the University of Kentucky campus (latitude 38.029151, longitude –84.509283) on a disturbed silt loam soil (pH 7.7; 33.4% sand, 50.9% silt, and 15.7% clay; 4.15% organic matter). We used 72 female plants, enough for 3 treatment timings, 2 neonicotinoids plus untreated checks, and 8 replicates per combination. Trees used in the trial were at least 2 m apart and buffered from one another by untreated trees.

Clethra alnifolia var. "Sixteen Candles" were obtained from a commercial nursery as 0.76-m tall shrubs in 18.9-L containers. They were transplanted to a site with tilled Maury silt loam soil (pH 6.0; 12.7% sand, 71.5% silt, and 15.9% clay; 5.6% organic matter) at the University of Kentucky's Spindletop Research Farm (latitude 38.129548, longitude –84.499315) on 19 September 2014. There were 54 total shrubs (3 timings × 3 treatments × 6 replicates), 4 rows each with 9 shrubs, with 2-m spacing between plants. The site was mulched, and the shrubs were irrigated weekly until 17 October 2014 to aid establishment, after which no further irrigation was applied until July 2016, when the shrubs were irrigated twice to alleviate effects of drought stress.

Neonicotinoid soil applications

Plants within each trial were treated with imidacloprid (Merit 2F; Bayer) or dinotefuran (Safari 20 SG; Valent U.S.A.) or left untreated as controls. Merit 2F is a liquid formulation containing 21.4% active ingredient (a.i.) with a label rate of 0.7–1.44 g a.i./0.305 m of plant height. Safari 20 SG is a water-soluble granule containing 20% a.i. with a label rate of 0.6 to 1.2 g a.i./0.305 m of plant height. Dinotefuran solutions were made using 709 mL distilled water and 126 g Safari 20 SG, and imidacloprid solutions were made using 603 mL distilled water and 106 mL Merit 2F, each within the products' range of label rates. The neonicotinoids were applied at equivalent dosages: 1.05 g a.i. (5.25 g Safari 20 SG, 87.5% of maximum label rate) for dinotefuran and 1.06 g a.i. (0.15 fl oz Merit 2F, 73.6% of maximum label rate) for imidacloprid, per 0.305 m of plant height. The *Ilex* had multiple trunks, so we used label rates for shrubs that are based on plant height as opposed to trunk diameter for single–trunk trees. The *Ilex* were treated with 210 mL of dinotefuran or imidacloprid solution to accommodate 2.13 m plant height, and *Clethra* were treated with 75 mL of dinotefuran or imidacloprid solution to accommodate 0.76 m plant height.

We originally intended to use a pressured soil injector of the type used by arborists to deliver the systemic pesticides into the soil but found that it failed to deliver consistent enough dosages for research purposes. We instead simulated soil injection by using a narrow-bladed hand trowel to open 6 holes (10.1 cm deep) in a circle approximately 15.1 cm from the base of each plant and injected equal portions of dinotefuran or imidacloprid solution into each hole with a 10-mL syringe. Treatments were not watered in, and there was no rainfall for at least 24 h after application. Treatment timings (Table 1) were either autumn (postbloom), spring (prebloom), or summer (postbloom).

TABLE 1: Dates on which woody plants received one-time soil treatment with imidacloprid or dinotefuran and on which nectar and foliage were sampled for residue analyses

		2015 Sampling		2016 Sampling	
Plant	Treatment date	Date sampled	Days since treatment[a]	Date sampled	Days since treatment[a]
Ilex	10 Nov 2014	2–12 June	204	9–16 May	546
	27 Mar 2015	2–12 June	67	9–16 May	409
	15 June 2015	—	—	9–16 May	329
Clethra	11 Nov 2014	22–31 July	253	20 Jul–3 Aug	617
	27 Mar 2015	22–31 July	117	20 Jul–3 Aug	481
	3 Aug 2015	—	—	20 Jul–3 Aug	352

[a]Days elapsed between systemic application and first day of sampling period. Each nectar harvest required several days to collect 100-μL samples per plant because of variation in flowering and the minute amounts of nectar per bloom.

Collecting nectar and foliage samples

Samples of nectar and foliage were collected from all plants during their respective bloom times in 2015 and 2016. Because of plant-to-plant variation in bloom times, each sampling period required several days to collect enough flowers to yield sufficient nectar for analysis. Sampling dates for *Ilex* were 2 to 12 June 2015 and 9 to 16 May 2016. *Clethra* was sampled 22 to 31 July 2015 and 20 July to 3 Aug 2016 (Table 1). Twigs with flowers were cut in the early morning, put in separate plastic bags for each plant, placed in coolers, and brought to the laboratory for processing the same day to maximize nectar yield. *Ilex* nectar was too viscous to collect via capillary tubes. To extract it, we collected twigs (2.5–5 cm), each bearing several flowers, removed the leaves, suspended the trimmed twigs from clips inside 15-mL centrifuge tubes, and spun them in a centrifuge (4000 rpm for 5 min). The expelled nectar was pipetted into 1.5-mL microcentrifuge tubes. *Clethra* nectar was collected directly from the flowers using 5-μL microcapillary tubes, which were then drained into microcentrifuge tubes as above. In 2015, only the minimum amount of leaves required for analysis (40–50 leaves) was collected from each plant during the same period as nectar extraction. This was done to prevent defoliating and damaging plants needed for the next year's residue analysis. In 2016, samples (50–100 g) of current-year (new) foliage were collected from all portions of each plant's canopy during the same period as nectar extractions. For *Ilex*, the broadleaf evergreen, samples of the previous years' (old) leaves were also collected. Nectar samples (in microcapillary tubes) and leaf samples (in plastic bags) were frozen at –80 °C, labeled with a random number code, and shipped on dry ice to the Valent Technical Center for residue analyses. Blanks and samples that had been spiked with known concentrations of imidacloprid or dinotefuran were also coded and included, ensuring a nonbiased double-blind analysis procedure.

Chemicals and reagents

All industrial sources for chemicals, reagents, supplies, and equipment described in this section and subsequent ones are based in the United States unless indicated otherwise. The analytical reference standards and deuterated compounds of imidacloprid (purity 99.9%) and imidacloprid-d_4 (purity 99.9%) were purchased from Sigma-Aldrich; imidacloprid olefin (purity 97.9%); 5-hydroxy-imidacloprid (purity 96.7%); imidacloprid olefin-$^{13}C_3$, ^{15}N, D (98.7%); and 5-hydroxy-imidacloprid-$^{13}C_3$, ^{15}N, D (90.7%) were obtained from Bayer CropScience; dinotefuran (purity 99.9%) was provided by Valent U.S.A.; and dinotefuran-d_3 (purity 98%) was obtained from C/D/N Isotopes. Acetonitrile (liquid chromatography–mass spectrometry [LC/MS] grade), methanol (LC/MS grade), and water (high-performance liquid chromatography [HPLC] grade) were purchased from VWR International. Formic acid (LC/MS grade) was obtained from Fisher Chemical. Magnesium sulfate and sodium chloride salts (each reagent grade) were purchased from Sigma-Aldrich. Peach blossom honey was purchased from Cooper Farms Country Store.

Preparation of standard solutions

Individual pesticide standard solutions (1 or 2 mg/mL for neat and deuterated) were prepared in acetonitrile. Imidacloprid, 5-hydroxy-imidacloprid, imidacloprid olefin, and dinotefuran standard stock solutions were further diluted with acetonitrile to prepare fortification standard solutions at concentration levels of 1000, 100, and 10 ng/mL. An internal standard mixture of imidacloprid-d_4; imidacloprid olefin-$^{13}C_3$, ^{15}N, D; 5-hydroxy-imidacloprid-$^{13}C_3$, ^{15}N, D; and dinotefuran-d_3 (2 ng/mL) was prepared in water/methanol (90/10, v/v). Calibration standards ranging from 500 to 0.05 ng/mL and containing 2 ng/mL of deuterated compounds were prepared in water/methanol (90/10, v/v) daily. Stock and fortification standard solutions were stored at –18 and 4 °C, respectively.

Nectar extraction

Received nectar samples were brought to ambient temperature and inspected for their quality. The nectar sample was briefly vortexed before extraction, and a 0.100-g subsample was weighed into a 1.8-mL autosampler vial. One milliliter of the internal standard solution (2 ng/mL in water/methanol [90/10, v/v]) was added to the sample. Further, the sample was vortexed and filtered through a Whatman 0.2 μm GD/X nylon filter disk (GE Healthcare Life Sciences) to remove any particulate materials. For

nectar samples with weight <0.1 g, required for residue analysis, the final volume of a sample was <1 mL, and the sample was centrifuged at 13 000 rpm for 5 min using a Sorvall Biofuge Pico centrifuge (Thermo Fisher Scientific) to remove particulates. The 2 techniques used to separate particulate materials from the samples did not affect the extraction efficiency of the studied compounds. In addition, sugar content in nectar samples was measured using the Eclipse Hand Held Refractometer model 45-81 and 45-82 (Bellingham & Stanley). For that, a drop (1–5 μL) of nectar sample was placed on the refractometer and the reading was rapidly performed to avoid alterations attributable to evaporation. Sugar content was measured in degrees Brix, where 1 °Bx is equal to 1 g sucrose in 100 g solution.

Leaf tissue extraction

In 2015, a subsample (2 g) of leaf tissues (or the whole sample if <2 g) was weighed directly into 50-mL polypropylene tubes prefilled with 2.8-mm ceramic beads (Omni). Ten milliliters of water acidified with 0.05% formic acid were added; then, the samples were shaken vigorously using an Omni Bead Ruptor 24 (Omni) at 3.7 motions/s for 60 s. The sample was then extracted with 10 mL of acetonitrile using the same procedure as for the water extraction. The extraction was followed by addition of 2.0 g of sodium chloride and 4.0 g of anhydrous magnesium sulfate salts. Samples were then centrifuged at 4000 rpm for 5 min using a Sorvall Evolution™ RC centrifuge (Thermo Fisher Scientific) to separate aqueous and organic phases. One milliliter of the organic supernatant was passed through a Strata C18-E cartridge (50 mg; Phenomenex) preconditioned with 1.0 mL of acetonitrile and rinsed with 0.5 mL of acetonitrile. The acetonitrile eluent was evaporated to dryness using a rotary vacuum evaporator and then reconstituted in 1.0 mL of internal standard solution (2 ng/mL). The sample was then filtered through a Whatman 0.2-μm GD/X nylon filter disk into an autosampler vial.

In 2016, leaf tissues were homogenized in the presence of dry ice using a coffee grinder (KitchenAid). Two grams of homogenized leaf sample were weighed into a 50-mL polypropylene centrifuge tube and extracted with 10 mL of water acidified with 0.05% formic acid and 10 mL of acetonitrile, followed by addition of 2.0 g of sodium chloride and 4.0 g of anhydrous magnesium sulfate salts, with the rest of the extraction procedure as described for the 2015 samples.

Liquid chromatography–tandem mass spectrometry analyses

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) analyses were carried out using a SCIEX API 4000 QTRAP triple-quadrupole/linear ion trap mass spectrometer operating in positive electron spray ionization mode (Applied Biosystems) coupled with an Agilent 1260 HPLC (Agilent Technologies) or a SCIEX 6500+ triple-quadrupole mass spectrometer (AB Sciex) equipped with an Agilent 1290 UPLC. Samples were separated using a reverse-phase Kinetex Biphenyl column (100 Å, 2.6 μm particle size, 100 × 2.1 mm; Phenomenex) coupled with a C18 security guard column (4.0 × 3.0 mm i.d. and 100 × 2.1 mm; Phenomenex) maintained at 30 °C. Mobile-phase solvents were water-acidified with 0.05% formic acid (A) and methanol acidified with 0.05% formic acid (B). The initial mobile-phase composition was 90% A and 10% B at a flow rate of 0.3 mL/min. The initial conditions were held for 0.5 min, followed by an increase to 50% B by 5.5 min, then an increase to 95% B by 10 min, and held for 4 min at 95% B. The analytical column was then brought back to initial conditions in 1 min and equilibrated for 5 min. The total run time was 20 min, and the injection volume was 30 μL for nectar samples and 10 or 30 μL for leaf samples. The mass spectrometer was operated using electrospray ionization in the positive ion mode. The mass spectrometer source was set at 400 °C, with nebulizer, curtain, and auxiliary gas at 50 (55 for 5-hydroxy-imidacloprid, imidacloprid olefin, and their counterparts), 30, and 40 units, respectively. The collision gas (N_2) was set at 10 units. High-purity nitrogen was used for all gases, and air was used as an auxiliary gas. The ion spray voltage was set at 5000 V (5500 V for 5-hydroxy imidacloprid, imidacloprid olefin, and their counterparts). Entrance potential was kept at 10 V (5 V for 5-hydroxy-imidacloprid). Two transitions, precursor and product ions, for each analyte were monitored. The monitored transitions and their mass spectrometer–specific parameters are indicated in Table 2. SCIEX Analyst Software 1.5 and 1.6.2 was used for data acquisition and quantitation.

TABLE 2: Monitored transitions and their mass spectrometer–specific parameters used in the residue analyses

Analyte	Q1	Q3	CXP (V)	CE (V)	DP (V)	Type
Dinotefuran	203.1	129.1	10	20	40	Quantitation
	203.1	157.0	10	20	40	Confirmation
Dinotefuran-d_3	206.2	132.2	10	20	40	Quantitation
5-Hydroxy-imidacloprid	272.2	191.2	5	30	50	Quantitation
	272.2	225.0	5	30	50	Confirmation
5-Hydroxy-imidacloprid-$^{13}C_3$, ^{15}N, D	277.0	196.1	15	30	20	Quantitation
Imidacloprid olefin	254.0	171.1	12	25	40	Quantitation
	254.0	205.0	12	25	40	Confirmation
Imidacloprid olefin-$^{13}C_3$, ^{15}N, D	258.9	176.1	15	30	40	Quantitation
Imidacloprid	256.0	209.1	15	30	20	Quantitation
	256.0	175.0	15	30	20	Confirmation
Imidacloprid-d_4	260.0	213.1	15	30	20	Quantitation

E = collision energy; CXP = collision cell exit potential; DP = declustering potential; Q1 = precursor ion; Q3 = quantification ion; V = volt.

TABLE 3: Average recoveries (and standard deviations) of analytes in nectar and leaf tissues

	Nectar			Leaf tissues			
Analyte	1.0 ng/g ($n=13$)	10.0 ng/g ($n=13$)	All levels ($n=26$)	5.0 ng/g ($n=5$)	50.0 ng/g ($n=5$)	10 000 ng/g ($n=3$)	All levels ($n=13$)
Dinotefuran	93 (10)	102 (5.4)	97 (9.2)	95 (4.3)	96 (1.7)	89 (4.2)	94 (4.2)
5-Hydroxy-imidacloprid	97 (16)	103 (7.4)	100 (13)	104 (2.0)	98 (2.3)	99 (6.2)	101 (4.1)
Imidacloprid olefin	106 (7.1)	101 (3.8)	104 (6.2)	94 (4.8)	95 (2.4)	101 (3.3)	96 (4.3)
Imidacloprid	102 (3.0)	105 (7.4)	103 (5.7)	100 (3.4)	99 (1.5)	116 (6.7)	103 (8.2)

Quality control

Methods for extracting nectar and leaf tissues were validated before sample analysis using diluted honey (25%, w/w) and cotton untreated control leaves. For nectar, the limit of detection (LOD) for all analytes was 0.5 ng/g, and the limit of quantitation (LOQ) was 1.0 ng/g. For leaf tissues, the LOQ for all analytes was 5.0 ng/g, and the LOD was 2.5 ng/g. Average recoveries and standard deviations for all analytes in nectar and leaf tissues are presented in Table 3.

All samples were analyzed in sets that included at least one untreated control sample and 2 control matrix samples fortified at the LOQ and 10 times the LOQ levels. Fortifications ranged from 1.0 to 5000 ng/g for nectar samples and from 5.0 to 10 000 ng/g for leaf samples. Average recoveries and standard deviations from concurrently analyzed fortified control samples of nectar and leaf samples are presented in Table 4.

Statistical analyses

Residue data were analyzed for main effects and interaction of treatment date, sample year, and (for *Ilex*) leaf age class using general linear model procedures (SAS, Ver 9.4; SAS Institute), with mean separation by least square means. Pearson correlation analysis was used to test for strength of correlation between imidacloprid and its metabolites in nectar and leaves. Data were analyzed separately by plant species and chemical. Data points below the LOD were entered as a zero, which is a conservative approach to handling data below detectable concentrations [45]. Data are presented as original means ± standard error (SE).

RESULTS

Imidacloprid, imidacloprid olefin, and 5-hydroxy-imidacloprid residues in Ilex nectar and leaves

Soil application of imidacloprid in autumn (postbloom, November 2014) or spring (prebloom, March 2015) resulted in mean concentrations of 276 and 166 ng/g imidacloprid, respectively, in *Ilex* nectar when the trees bloomed in spring 2015 (Table 5), with no significant difference between the treatment dates (Table 6 and Figure 1A). Those residue levels declined by approximately 88 and 79%, respectively, by the time the trees bloomed again in spring 2016. Trees treated in summer (postbloom, June 2015) had only approximately 8 ng/g imidacloprid in their 2016 nectar, levels comparable to or lower than those present in the second year of bloom for the other 2 timings (Figure 1A). Residues of the metabolites imidacloprid olefin and 5-hydroxy-imidacloprid in *Ilex* nectar (Table 5) were highly correlated with those of imidacloprid ($r=0.98$ and 0.96 for 2015 and 2016, respectively; $p<0.0001$; $n=54$). Sugar concentrations in *Ilex* nectar (Table 5) ranged from 42 to 78 °Bx in 2015 and from 13 to 66 °Bx in 2016.

Imidacloprid residues in *Ilex* leaves followed a similar pattern to those in the nectar, with no significant difference between treatment dates but a significant decline between 2015 and 2016, especially in the newly flushed (current year) leaves (Table 6 and Figure 1B). Within treatment dates and years, imidacloprid concentrations in new leaves were 28 to 90 times higher than those in nectar. Summer treatment resulted in the least amount of imidacloprid in the following spring's foliage (Figure 1B). Imidacloprid olefin and 5-hydroxy-imidacloprid residues were highly correlated with imidacloprid levels in foliage ($r=0.86$ and 0.88 for 2015 and 2016; $p<0.0001$; $n=137$ for combined new and 1-yr-old leaves).

TABLE 4: Average recoveries (and standard deviations) of analytes from concurrently analyzed fortified control nectar and leaf samples

	Nectar		Leaf tissues	
Analyte	n	All levels	n	All levels
Dinotefuran	28	105 (10.6)	72	82.4 (7.5)
5-Hydroxy-imidacloprid	28	109 (10.4)	64	100 (8.7)
Imidacloprid olefin	28	112 (16.9)	69	101 (10.6)
Imidacloprid	36	103 (14.9)	71	79.5 (29.0)

Imidacloprid, imidacloprid olefin, and 5-hydroxy-imidacloprid residues in Clethra nectar and leaves

Uptake and dissipation of imidacloprid residues in *Clethra* nectar followed a similar pattern to residues in *Ilex* nectar (Table 6 and Figure 1C). There was no significant difference between the autumn (postbloom, November 2014) and spring (prebloom, March 2015) treatment dates, both of which resulted in high levels in summer 2015 nectar. Those levels declined by 83 to 85% when the trees bloomed again in the summer of 2016 (Figure 1C). As with the *Ilex*, autumn treatment resulted in relatively low residue levels in nectar the following year (Figure 1C). Imidacloprid olefin and 5-hydroxy-imidacloprid residues (Table 5) were highly correlated with imidacloprid residues in *Clethra* nectar ($r=0.82$ and 0.94 for 2015 and 2016, respectively; $p<0.001$; $n=65$ in each

TABLE 5: Mean (range) concentrations of sugar (°Bx),[a] imidacloprid (nanograms per gram), its metabolites imidacloprid olefin and 5-hydroxy-imidacloprid in nectar of *Ilex* (Foster holly) or *Clethra* (summersweet) following systemic treatment by soil injection[b]

Plant species	Application timing	Year collected	Sugar	Imidacloprid	Imidacloprid olefin	5-Hydroxy-imidacloprid
Ilex	Nov 2014	2015	69 (66–74)	276 (122–560)	55 (22–123)	22 (7–54)
		2016	33 (13–51)	32 (11–85)	4 (2–10)	3 (1–8)
	Mar 2015	2015	60 (42–78)	166 (1–459)	32 (0–81)	9 (0–23)
		2016	36 (15–66)	52 (34–84)	10 (4–18)	5 (3–8)
	Jun 2015	2016	32 (14–55)	8 (6–15)	1 (1–2)	2 (0–2)
Clethra	Nov 2014	2015	12 (3–24)	515 (213–1017)	55 (16–117)	69 (37–136)
		2016	15 (15–17)	86 (16–192)	40 (13–65)	27 (8–51)
	Mar 2015	2015	11 (1–26)	381 (172–668)	40 (14–71)	46 (29–70)
		2016	13 (11–17)	60 (25–107)	28 (19–35)	23 (17–30)
	Aug 2015	2016	13 (10–18)	31 (5–47)	16 (3–21)	12 (2–16)

[a]One °Bx is equal to 1 g sucrose in 100 g solution.
[b]Imidacloprid olefin and 5-hydroxy-imidacloprid residues were highly correlated with imidacloprid residues: Pearson correlation coefficients $r = 0.98$ and 0.95, respectively, for *Ilex*; $r = 0.82$ and 0.94 for *Clethra*, all $p < 0.001$.

year). Residues of imidacloprid in *Clethra* leaves followed a similar pattern to those in *Clethra* nectar, with no differences between autumn and spring treatment timings and 77 to 84% declines between 2015 and 2016 (Table 6 and Figure 1D). Concentrations were approximately 50-fold higher in leaves than in nectar. Imidacloprid olefin and 5-hydroxy-imidacloprid residues were highly correlated with imidacloprid residues in *Clethra* leaves ($r = 0.97$ and 0.97 for 2015 and 2016, respectively; $p < 0.0001$, $n = 69$ in each year). Sugar concentrations in *Clethra* nectar (Table 5) ranged from 1 to 26 °Bx in 2015 and from 10 to 18 °Bx in 2016.

Dinotefuran residues in Ilex nectar and leaves

Uptake and dissipation of dinotefuran residues in *Ilex* nectar (Figure 2A) showed a different pattern from imidacloprid. For dinotefuran, there were significant main effects for treatment date and year (Table 6). Soil injection in autumn (postbloom, November 2014) or spring (prebloom, March 2015) resulted in high concentrations in the 2015 nectar (Figure 2A), especially from the spring application. However, those residues were nearly gone (≤3 ng/g) by the time the trees bloomed again in 2016. The summer (postbloom, June 2015) application resulted in high dinotefuran concentrations in nectar the following spring (Figure 2A). Mean (range) sugar concentrations in *Ilex* nectar were 62 (59–64) °Bx and 68 (64–74) °Bx in 2015 for autumn and spring applications, respectively. Sugar concentrations in 2016 were 48 (42–54), 27 (5–66), and 46 (33–57) °Bx for autumn, spring, and summer applications, respectively.

Dinotefuran residues in *Ilex* leaves also showed significant main effects for treatment date and year sampled (Table 6 and Figure 2B). November 2014 and March 2015 applications resulted in mean concentrations of 4751 and 6287 ng/g in 1-yr-old and new leaves, respectively, sampled coincident with bloom in May 2015; but those levels had declined by >99% by spring 2016 (Figure 2B). In contrast, residues from the summer treatment timing were still present at high amounts in foliage sampled in spring of the following year.

TABLE 6: Summary of analysis of variance for effects of treatment date (November 2014, March 2015, or June/August 2015), year sampled (2015 or 2016), and leaf age class (current or 1-yr-old leaves, *Ilex* only) on residue levels (nanograms per gram) of imidacloprid or dinotefuran in nectar or foliage of 2 species of woody landscape plants

		Residues in *Ilex* nectar				Residues in *Clethra* nectar				Residues in *Ilex* foliage				Residues in *Clethra* foliage			
		Imidacloprid		Dinotefuran		Imidacloprid		Dinotefuran		Imidacloprid		Dinotefuran		Imidacloprid		Dinotefuran	
Source	*df*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*	*F*	Pr > *F*
Trt date (T)[a]	2	0.69	0.51	8.24	0.002	1.21	0.003	6.58	<0.005	1.47	0.24	12.0	<0.0001	1.14	0.33	16.7	<0.0001
Year (Y)[b]	1	14.7	<0.001	6.70	0.02	45.6	<0.001	5.29	0.03	27.0	<0.001	4.38	0.04	45.7	<0.0001	19.3	0.001
T×Y	1	1.32	0.26	2.96	0.10	0.95	0.34	2.96	0.10	2.1	0.15	2.14	0.15	1.82	0.19	9.1	0.005
Leaf age (A)[c]	1	–	–	–	–	–	–	–	–	0.48	0.49	0.35	0.56	–	–	–	–
T×A	2	–	–	–	–	–	–	–	–	0.46	0.46	1.08	0.34	–	–	–	–
Y×A	1	–	–	–	–	–	–	–	–	1.46	1.46	0.27	0.60	–	–	–	–
T×Y×A	1	–	–	–	–	–	–	–	–	0.05	0.05	0.01	0.91	–	–	–	–

[a]Treatment dates were November 2014, prebloom (March 2015) or postbloom (15 June or 3 August for *Ilex* or *Clethra*, respectively).
[b]Nectar and foliage were sampled during bloom in 2015 or 2016.
[c]For *Ilex*, a broad-leaved evergreen, both current year and 1-yr-old leaves were sampled.
A = leaf age; T = treatment date; Y = year.

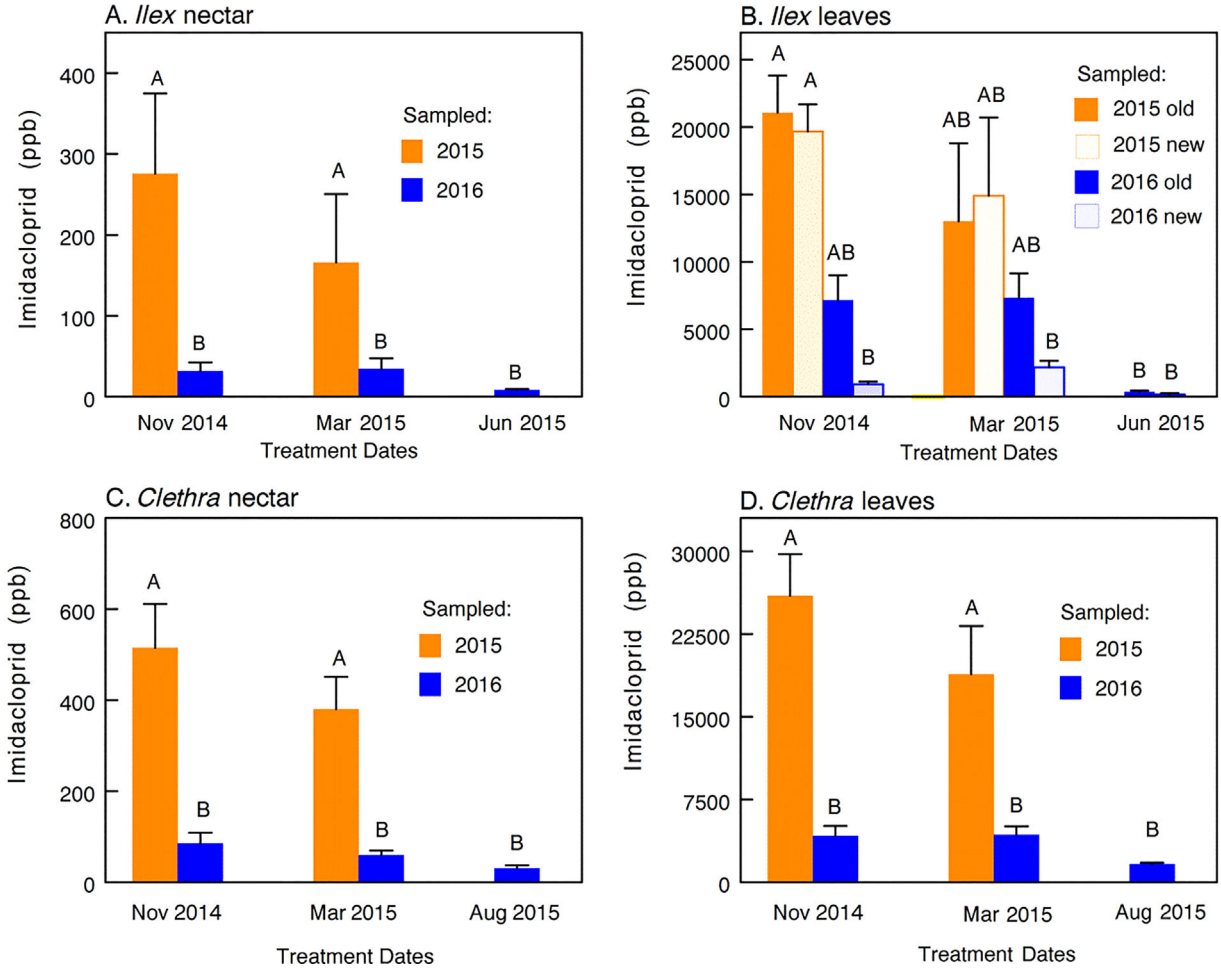

FIGURE 1: Mean (± standard error) concentrations (nanograms per gram) of imidacloprid in floral nectar of *Ilex* × *attenuata* (Foster holly) and *Clethra alnifolia* (summersweet) following systemic soil treatment in autumn (postbloom, November 2014), spring (prebloom, March 2015), or summer (postbloom, June or August 2015 for *Ilex* and *Clethra*, respectively). $n \geq 3$ for all *Ilex* treatments, $n \geq 5$ for all *Clethra* treatments. Bars not topped by the same letter differ significantly (least squares means, $p < 0.05$). Analysis of variance results are summarized in Table 6.

Dinotefuran residues in Clethra nectar and leaves

Patterns of uptake and dissipation of dinotefuran in *Clethra* nectar and leaves were similar to *Ilex* (Figure 2C and D). Main effects for treatment date and sample year both were significant (Table 6). Compared with the spring treatment timing, application in autumn resulted in lower dinotefuran residues in spring 2015 nectar and leaf tissue. Treating in summer 2015 resulted in high levels of dinotefuran in nectar and foliage the following summer (Figure 2C and D). Mean (range) sugar concentrations in *Clethra* nectar were 11 (2–26) and 18 (3–54) °Bx in 2015 for autumn and spring applications, respectively. Sugar concentrations in 2016 were 16 (15–17), 15 (14–19), and 14 (12–18) °Bx for autumn, spring, and summer applications, respectively.

DISCUSSION

The present study shows that soil application of imidacloprid or dinotefuran at landscape label rates can result in residues in nectar of woody landscape plants that exceed concentrations shown in semifield and laboratory studies to adversely affect individual- and colony-level traits of bees [32–37] as well as the no- and lowest-observed-adverse-effect concentrations (25 and 50 ng/g, respectively) for honeybee colonies [46]. Those levels, particularly in the first spring after autumn or spring application, were also much higher than the 1 to 10 ng/g typically found in nectar of field crops such as canola or sunflower grown from treated seed [10,33].

Neonicotinoid label rates for soil application to woody landscape plants are much higher on a per-plant basis than those used to protect field crops [47]. Those rates are broad, in part because of the variety of pests and plant species in urban landscapes. Lowering the label rates may reduce risk to pollinators, but it is unknown if doing so would still provide control of key pests, especially because uptake of residues will vary depending on plant species, size, and health, as well as soil type and environmental conditions.

Reported half-lives of neonicotinoids in soils vary greatly across soil types and conditions [10]. Calculated half-lives for imidacloprid range from 107 to 1250 d, depending on soil conditions [10,33]. Once it is absorbed by a tree or shrub, imidacloprid may be relatively stable. In hemlock

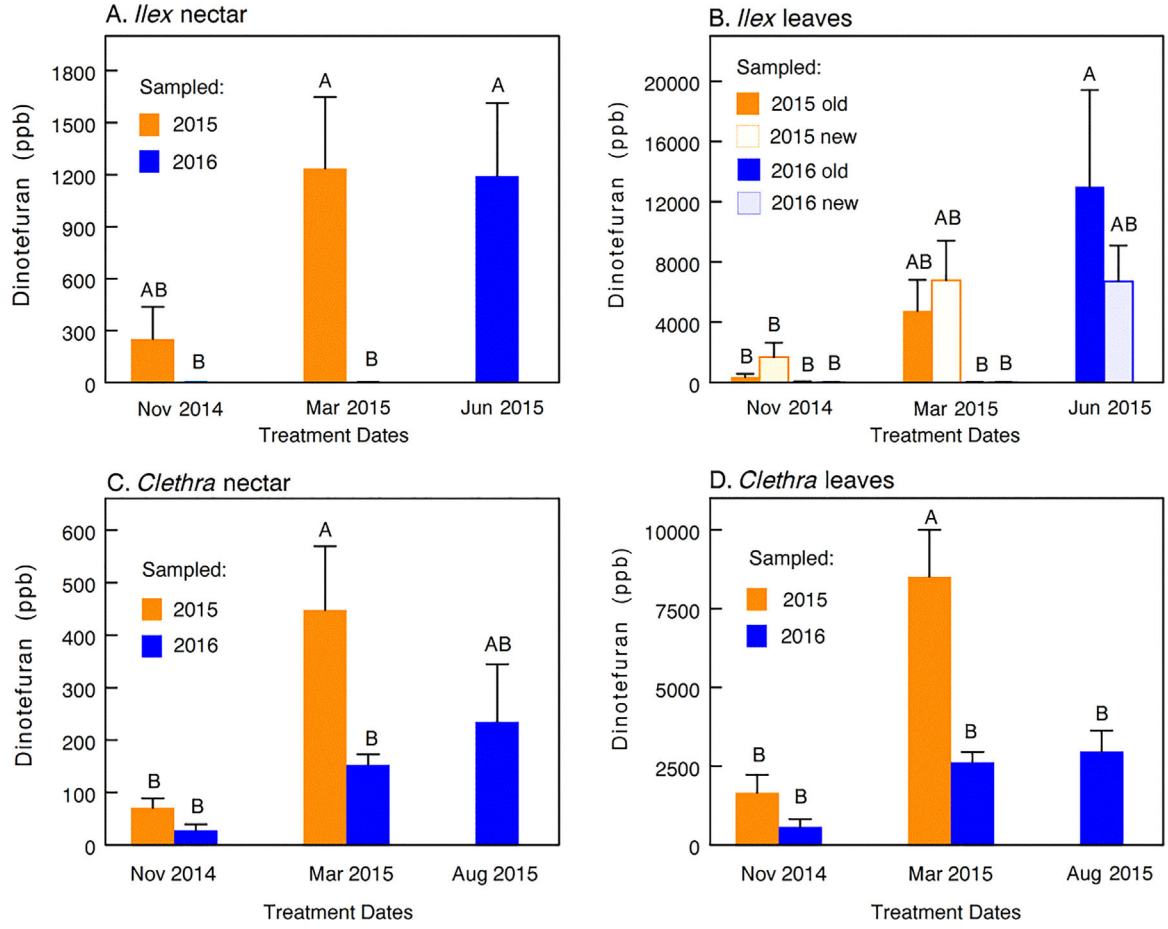

FIGURE 2: Mean (±standard error) concentrations (nanograms per gram) of dinotefuran in leaves of *Ilex* × *attenuata* (Foster holly) and *Clethra alnifolia* (summersweet) following systemic soil treatment in autumn (postbloom, November 2014), spring (prebloom, March 2015), or summer (postbloom, June or August 2015 for *Ilex* and *Clethra*, respectively). Bars not topped by the same letter differ significantly (least squares means, $p < 0.05$). Analysis of variance results are summarized in Table 6.

(*Tsuga canadensis*), an evergreen species, imidacloprid concentrations in foliage peak approximately 9 to 15 mo after soil application; but concentrations of its metabolite imidacloprid olefin continue to rise for as long as 3 yr after treatment [48]. The multiyear suppression of hemlock woolly adelgid provided by such treatments suggests multiple years of mobilization of imidacloprid and imidacloprid olefin to new growth [4,39]. In contrast, most of the accumulation of imidacloprid into leaves of ash (*Fraxinus* spp.), which is deciduous, occurs during the growing season immediately after treatment, with little remobilization the following year [9,49].

Dinotefuran is approximately 80 times more soluble than imidacloprid, and its soil sorption coefficient is >10-fold lower [15]. Its estimated half–life in soil under field conditions is approximately 75 d [10]. Studies comparing the 2 compounds' metabolism in ash [50], hemlock [40], walnut [43], and avocado [41] suggest that dinotefuran tends to be translocated more quickly than imidacloprid; but it may also undergo relatively more rapid degradation in woody plant tissues.

We hypothesized that differences in systemic mobility and persistence between imidacloprid and dinotefuran would allow one or the other of the compounds to be applied in autumn or summer with minimal transference of residues into floral resources. Ideally that would allow landscape managers to match product and timing to control pests with minimal hazard to bees.

Summer 2015 (early postbloom) applications of imidacloprid resulted in relatively low concentrations in 2016 nectar of both plant species. If that pattern holds for other woody plant species, treating with imidacloprid soon after bloom may allow for control of pests such as aphids, leaf-feeding beetles, or scale insects with minimal hazard to pollinators. However, summer application also gave relatively little transference into new foliage, which could limit effectiveness against pests such as psyllids that feed on and distort expanding new leaves in spring. Autumn and spring imidacloprid treatments resulted in high concentrations in foliage and nectar, including some remobilization into new growth and floral resources in the second year. Because the plants were dormant when treated in November, there was likely little uptake from the soil until bud-break the following spring. Thus, both treatments would have had similar time for the uptake of the relatively slow-mobilizing imidacloprid once the plants became metabolically active in spring.

Dinotefuran showed a different pattern of uptake and dissipation from imidacloprid. In *Ilex*, autumn 2014 or spring

2015 application was followed by high nectar residue levels when the plants bloomed in 2015 but almost no deposition into nectar or foliage the following year. Summer application, however, resulted in unexpectedly high deposition of residues in nectar and in both new and 1-yr-old leaves the following spring. Relationships between application timing and dinotefuran residues in *Clethra* were generally similar to the patterns in *Ilex*. Given the relatively short estimated half-life of dinotefuran in field soil [10], it seems unlikely that enough of the compound would persist for 11 mo in the soil to account for the high 2016 residue levels. Instead, residues taken up by the plants during the 2015 growing season were likely still present in the woody tissues or 1-yr-old leaves and remobilized to both nectar and new leaves during leaf flush and flowering the following spring.

Neonicotinoids circulate mainly via xylem transport [10], so their uptake in plants is greatest during periods of active growth and transpiration. At the time of sampling in spring 2016, plants treated in autumn 2014 and spring 2015 had undergone 2 separate annual leaf flushes, whereas those treated in summer 2015 had undergone only one. We hypothesize that residues in summer-treated plants experienced less dilution and degradation in foliage over the course of one leaf flush versus 2, leaving more available for remobilization into nectar. It seems less likely that dinotefuran remained in the soil because of the steep drop-offs we observed in second-year residues. Compared with imidacloprid, much less is known about the fate of dinotefuran in woody plant tissues. Our data suggest that dinotefuran may be more persistent in plants than is generally believed.

Our data indicate that even if label directions to "make application prior to anticipated pest infestation to achieve optimum levels of control" (Merit 2F and 75 WP labels) or "time applications to coincide with when most vulnerable pest life stage is present on plants" (Safari 20 SG label) are followed, use of those products on bee-attractive woody landscape plants could result in residue levels in floral resources higher than those known to adversely affect bees. Likewise, instructions on the US Environmental Protection Agency's Bee Advisory Box such as "Do not apply while bees are foraging," "Do not apply to plants that are flowering," and "Only apply after all flower petals have fallen off" would not necessarily alleviate potential risk on such plants.

Insecticide hazard to pollinators depends on the toxicity of the pesticide, the extent of exposure, and the effects of that exposure on individual or colony fitness [35]. Interpreting the present results in the context of pest management and pollinator protection is complex because no regulatory limit currently exists for either dinotefuran or imidacloprid residues in nectar of woody landscape plants. Furthermore, little is known about bees' exposure to treated plants in landscape settings.

Most bee species in urban landscapes are polylectic, collecting pollen and nectar from a variety of flowering weeds and other spontaneous plants, as well as from ornamental forbs, shrubs, and trees [23,29,51]. Such dietary diversity would likely dilute the effects of occasional sublethal exposure to neonicotinoid-treated plants. Exposure will also be affected by the percentage of bee-attractive plants in a given neighborhood that are treated with neonicotinoids, which is likely to be low, and the length of time that those plants are in bloom. Bee colonies in orchards or field crops may be exposed to monocultures of treated plants for the duration of flowering, which in some cases (e.g., canola or oilseed rape) can last as long as 6 wk [52]. In contrast, individual woody landscape plants tend to bloom and attract bees for shorter periods, often no more than 1 to 2 wk (authors' observations).

Systemic nitroguanidine neonicotinoids are versatile tools for managing insect pests, including invasive species of trees and shrubs, but guidelines are needed to help land care professionals and homeowners use them without harming bees and other pollinators. The present results indicate that residues in nectar are likely to intoxicate individual pollinators foraging exclusively on treated woody plants. Therefore, a recommendation for integrating pest and pollinator management is to avoid their use on bee-attractive trees and shrubs unless there is no other way to prevent significant pest damage to such plants. Future work is needed to define the percentage of floral resources that systemically treated plants represent in urban landscapes. Without such data, it is difficult to draw conclusions about the impact of these treatments on pollinator health at the landscape level.

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Data Availability—Data, associated metadata, and calculation tools are available from the corresponding author (dapotter@uky.edu).

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