

Dynamic Behavior of Systemic Insecticide Residues in Snapdragons

Matthew C. Havers

Department of Plant Biology, Rutgers, The State University of New Jersey, IR-4 Project, 59, Dudley Road, New Brunswick, NJ 08901, USA

Lea Corkidi

University of California Cooperative Extension, 9335 Hazard Way, Suite 201, San Diego, CA 92123, USA

Elizabeth Leonard

Department of Plant and Environmental Sciences, Clemson University, Clemson, SC 29634, USA

Cristi L. Palmer

Department of Plant Biology, Rutgers, The State University of New Jersey, IR-4 Project, 59, Dudley Road, New Brunswick, NJ 08901, USA

Nishanth Tharayil

Department of Plant and Environmental Sciences, Clemson University, Clemson, SC 29634, USA

James A. Bethke

University of California Cooperative Extension, 9335 Hazard Way, Suite 201, San Diego, CA 92123, USA

Keywords. *Antirrhinum majus*, foliar spray, liquid chromatography–mass spectrometry, pollinator, soil drench

Abstract. Pesticide application is used in horticulture to reduce plant damage from organisms such as insects and mites. Systemic insecticides are highly efficacious and readily taken up by plant tissues. However, pesticide-treated plants may impose risks to nontarget insects or other organisms within ecosystems. In this study, insecticide residues in nectar, leaves, and flower petals of the horticulturally significant herbaceous annual snapdragon, *Antirrhinum majus* (Lamiales: Plantaginaceae), were assessed at two locations over several weeks following foliar and drench treatment with five systemic insecticides. Concentrations of the insecticides were determined by liquid chromatography–mass spectrometry. The independent effects *Application Method*, *Application Rate*, and *Time* were statistically significant among all active ingredients in the three matrices in both sites in California (CA) and New Jersey (NJ). The interaction effects were also generally statistically significant in the CA site but less consistently so in the NJ site, dependent on the active ingredient and matrix. Post hoc analyses found the highest residue concentrations in leaves and the lowest in nectar, a trend generally consistent over time regardless of active ingredient for both the CA and NJ sites. The results of this study are discussed in the context of conserving pollinators and other beneficial insects. It is recommended that similar studies should be implemented in different geographical regions and climates, along with multiyear studies for perennial ornamental plants.

Systemic insecticides are useful for pest management in the ornamental horticulture industry because of their efficacy and their ability to protect most or all parts of the treated plant. However, residues of these insecticides may also translocate to pollen and nectar, potentially exposing economically or ecologically important pollinators and other nontarget organisms, to residues. For example, residues of cyantraniliprole and one of its metabolites (J9Z38) at levels above 5 mg/kg eliminated the integrity of cell structure in earthworms due to oxidative stress (Zhang et al. 2020). A summary of peer-reviewed literature from 2009 to 2019 indicated that 90% of the

studies showed negative effects of chronic sublethal doses of neonicotinoids on pollinator health, ranging from impairment of brood development and foraging activity to neurological damage (Lu et al. 2020).

Concentrations of neonicotinoids found in plant tissues between 5 and 10 parts per billion (ppb) are considered sufficient to protect from insect pests (Byrne and Toscano 2006; Castle et al. 2005); however, nontarget insect species, such as pollinators, may also be sensitive to these insecticides at lower amounts. For example, the LC₅₀ (the concentration of a certain molecule that will kill 50% of the test subjects) of imidacloprid and clothianidin is

between 4 and 5 ng for individual *Apis mellifera*, which when ingested is 0.010% of the mass required of DDT to yield the same result (Suchail et al. 2000). The 48-hour oral LD₅₀ of *Bombus impatiens* (eastern bumble bee) was >1.7 µg of flupyradifurone (a butenolide), >0.54 µg of cyantraniliprole (a diamide), and 0.0012 µg of thiamethoxam (a neonicotinoid) (Mundy-Heisz et al. 2022). Thiamethoxam can affect egg development, colony initiation, worker efficiency and memory, and male size through ingestion at concentrations of 2.4 ppb and 1 ppm on bumble bees and honeybees, respectively (Baron et al. 2017; Stanley and Raine 2017; Stanley et al. 2015a, 2015b). Multiple pesticides contained in either plant tissue or nectar can potentially increase risk. Imidacloprid and thiamethoxam together increased risk to honeybee colony health through exposure to contaminated pollen and honey (Sanchez-Bayo and Goka 2014). Insecticides can act synergistically with triazole fungicides to create greater risk to pollinator health (Sanchez-Bayo and Goka 2014). Timing of exposure to low levels of neonicotinoid insecticides affects honeybee colonies differently with no immediate negative effects early in the season but exposure increased brood failure later in the season, which affects colony health during overwintering (Dively et al. 2015).

A large variety of ornamental plants are found in urban systems (Loram et al. 2008). Research into their attractiveness to pollinators and impacts on pollinator health has increased in recent years, but their contribution to pollinator conservation is currently in question, with factors such as morphological variability among cultivars, nectar rewards, and environment contributing to the debate (Erickson et al. 2020; Garbuzov and Ratnieks 2014; Lowenstein et al. 2019; Rollings and Goulson 2019). Insecticides are often used on ornamental plants in production and in landscapes. Several studies on ornamental plants have documented the presence of neonicotinoids and organophosphates in leaf and/or pollen at levels that could pose health risks for bees (Lentola et al. 2017; Toumi et al. 2016). Although pollen and nectar are the most commonly analyzed matrices for pesticide residues, very few studies include flower petals and leaves, two tissues underrepresented in ornamental plant residue studies.

Snapdragons, widely grown in commercial and residential gardens in the United States, are attractive to pollinators and specifically known to be visited by large-bodied and long-tongued bees (Vargas et al. 2017). The purpose of this study was to determine concentrations of the systemic insecticides, namely dinotefuran, imidacloprid, thiamethoxam, flupyradifurone, and cyantraniliprole, in the nectar, flower petals, and leaves of snapdragons and determine whether they vary according to application method (foliar or drench), application rate (high or low label rates), and time (week after treatment). In this study, two experiments were conducted at different sites using two different cultivars, housing systems, and environmental

Table 1. Pesticides, active ingredients, and doses used for foliar and drench treatment applications.

Product	Active ingredient	Application method	Dose	Amount per 100 gal water	Amount of product used per liter of water	Amount of product per container NJ (mL)	Amount of product per container CA (mL)
Marathon	Imidacloprid	Foliar	Low	0.85 fl oz	0.07 mL/L	0.45	0.05
			High	1.7 fl oz	0.13 mL/L	0.85	0.09
		Drench	Low	4.03 fl oz	0.31 mL/L	1.99	0.2
			High	8.06 fl oz	0.63 mL/L	4.05	0.41
BY102960 50 SL (Altus)	Flupyradifurone	Foliar	Low	7 fl oz	0.55 mL/L	3.54	0.36
			High	14 fl oz	1.09 mL/L	7.01	0.72
		Drench	Low	14 fl oz	0.07 mL/L	0.45	0.05
			High	28 fl oz	0.15 mL/L	0.97	0.10
Safari 20.0 W/W SG	Dinotefuran	Foliar	Low	4 oz	0.30 g/L	1.93	0.2
			High	8 oz	0.6 g/L	3.86	0.39
		Drench	Low	12 oz	0.9 g/L	5.79	0.59
			High	24 oz	1.80 g/L	11.6	1.18
Flagship 25WG	Thiamethoxam	Foliar	Low	2 oz	0.15 g/L	0.97	0.10
			High	8.5 oz	0.64 g/L	4.12	0.42
		Drench	Low	4 oz	0.30 g/L	1.93	0.2
			High	8.5 oz	0.64 g/L	4.12	0.42
Mainspring GNL	Cyantraniliprole	Foliar	Low	1 fl oz	0.08 mL/L	0.51	0.05
			High	16 fl oz	1.25 mL/L	8.04	0.82
		Drench	Low	6 fl oz	0.47 mL/L	3.02	0.31
			High	12 fl oz	0.94 mL/L	6.05	0.62

CA = California; NJ = New Jersey.

growing conditions. Exploration of residue consistency among different growing practices allows for a larger population of horticulturists to use the results of this study when making decisions on insecticidal applications.

Materials and Methods

Greenhouse experiments were conducted in NJ and southern CA, USA, to analyze the residues of different insecticides in the nectar, petals, and leaves of the common snapdragon (*Antirrhinum majus* L.). Although the same rates of insecticides were used, snapdragon cultivar, pot size, nectar collection method, and sample collection timings varied between these two locations.

Pesticide treatments

All plants were treated once flower buds were forming, ~2 months after planting. Treatments [Marathon II (imidacloprid), Altus (flupyradifurone), both Bayer Crop Science LP, Clayton, NC, USA; Safari 20 SG (dinotefuran), Valent USA Corporation, Leland, MS, USA;

and Flagship 25WG (thiamethoxam), Mainspring GNL (cyantraniliprole), both Syngenta Crop Protection LLC, Greensboro, NC, USA] were applied either as media drenches or as foliar sprays at high and low rates (Table 1). Foliar sprays were applied to the point of runoff using a spray bottle or backpack sprayer with three-nozzle boom; media drenches were applied to pots at volumes based on pot size. Plants in the control group received only a water spray.

Greenhouse experiment in southern California

In southern California, snapdragon ‘Sonnet White’ plants were grown in a greenhouse at the University of California South Coast Research and Extension Center, in Irvine, CA. From Mar to Jul 2019, average maximum and minimum temperatures were 28 and 18 °C.

Snapdragon ‘Sonnet White’ seedlings (Ball Horticultural Company, West Chicago, IL, USA) were transplanted into plastic containers (656 mL, 25 cm deep, 6.4-cm-diameter deepots; Steuwe and Sons, Corvallis, OR, USA) filled with a standard nursery mix composed of 55% to 60% white peat, 10% to 20% perlite, and 25% to 35% aged fine bark (SunGro Metro Mix 838; Crop Production Services, Inc., Goleta, CA, USA). Plants were hand-watered as needed and fertilized with 0.25 tsp of Osmocote 14N–14P–14K controlled release fertilizer, 2 weeks after planting. Foliar and drench application treatments were applied on 21 May 2019; drenches were made using 20 mL of diluted insecticide per pot. Forty-five plants were used for each treatment, with five plants per replicate and nine replications per treatment randomly arranged in a completely randomized design and maintained on greenhouse benches.

Three composite samples of three to five leaves and 30 to 45 flowers obtained from 15 different plants each were collected 2, 4, 6, and 8 weeks after treatment. Leaves were

immediately frozen, and flowers were rinsed and placed in plastic bags and stored in a refrigerator until nectar extraction using microtubes. Nectar was pipetted into microcentrifuge tubes and frozen. Samples of nectar, leaves and petals were shipped on dry ice to Clemson University for pesticide residue analysis. On arrival, samples were stored at –80 °C until analysis.

Greenhouse experiments in NJ

In NJ, snapdragons ‘Sonnet Yellow’ were grown in three heated, plastic-covered hoop-houses at the Rutgers, State University of New Jersey Specialty Crop Research Center, in Cream Ridge, NJ, USA. Two separate trials were conducted from Dec 2019 to Mar 2020 and Dec 2020 to Mar 2021. Over the entire trial period in both trials, the average humidity in the hoop-houses was 75% and the average maximum and minimum temperatures were 27° and 18 °C, respectively. Snap-dragon seedlings (KubePak, Allentown, NJ, USA) were transplanted into plastic containers (class 600 pots; Hummert International, Earth City, MO, USA) filled with a standard nursery mix composed of 55% to 60% white peat, 10% to 20% perlite, and 25% to 35% aged fine bark (SunGro Professional Growing Mix; Sungro Horticulture, Agawam, MA, USA). Plants were overhead-irrigated as needed and fertilized with 15N–9P–12K Osmocote Plus fertilizer 2 to 5 d after planting; 25 to 30 plants were used per treatment, with single-plant replicates. Treatments were arranged in a randomized block design with 10 plants of each treatment per heated hoop-house (block). Treatments were applied once in each trial on 17 Jan 2018 and 7 Jan 2019. Drench applications were made using 4 fl oz (118.294 mL) of prepared solution per pot. Foliar sprays were applied to drip with a CO₂-powered backpack sprayer with a three-nozzle boom.

Three composite samples of four to five leaves, petals from five flowers, and nectar pipetted from flowers were obtained from each

Received for publication 22 Jan 2024. Accepted for publication 3 Apr 2024.

Published online 8 May 2024.

This research was supported by USDA-NIFA SCRI Grant 2016-51181-25399: “Protecting pollinators with economically feasible and environmentally sound ornamental horticulture”.

The Rutgers team thanks Carolina Roe-Raymond, Jackie Cavaliere, and David Bodine. The University of California Cooperative Extension team thanks Darren L. Haver, Randall E. Musser, Chris Martinez, Arnaldo Miranda-Urrea, Kathie Burns, Gloria Chen, Chris Drees, Nancy Hamilton, Louise Karkoutli, Carla Merrigan-Ward, Shaye Shayegani, Gale Slagle, Co Wilkins, Annika Nabors, Maryam G. Ibrahim, and Bryan Vander Mey.

C.L.P. is the corresponding author. E-mail: clpalmer@njaes.rutgers.edu.

This is an open access article distributed under the CC BY-NC-ND license (https://creativecommons.org/licenses/by-nc-nd/4.0/).

treatment in each block 2, 6, and 10 weeks after application. Leaves and flower petals were immediately frozen. Collected nectar was pipetted into microcentrifuge tubes and frozen in batches; if less than 0.5 mL was collected, samples were frozen, and collections continued daily for 1 to 4 d per plot until at least 1 mL was collected unless insufficient flowers were available. Samples of nectar, leaves, and flower petals were shipped via semifreezer truck (ACDS, North Rose, NY, USA) to Clemson University for pesticide residue analysis. On arrival, samples were stored at -80°C until analysis.

Pesticide residue methods

Chemicals. Chemical standards for the stable isotope-labeled (SIL) pesticides, dinotefuran- $(^{13}\text{C}_5)$, thiamethoxam- $(^{13}\text{C}_4, ^{15}\text{N})$, clothianidin- $(^{13}\text{C}_4, ^{15}\text{N})$, and imidacloprid- (d_4) , were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Custom-synthesized flupyradifurone- (d_5) and cyantraniliprole- (d_3) standards were purchased from Clearysynth (Brampton, Ontario, Canada). A stock solution mixture of six SIL standards at 1 $\mu\text{g/mL}$ was prepared in acetonitrile and stored at -20°C . High-purity analytical standards for dinotefuran, thiamethoxam, clothianidin, imidacloprid, flupyradifurone, cyantraniliprole, and chlorantraniliprole were purchased from Chem Service, Inc. (West Chester, PA, USA) and imidacloprid-olefin (imidacloprid metabolite) was purchased from Sigma-Aldrich (St. Louis, MO, USA). A stock solution of each of the eight standards and metabolite (1 $\mu\text{g/mL}$) was prepared in acetonitrile and stored at -20°C . Chlorantraniliprole was added to the standard mixture as a quality control check for reagent contamination during the pesticide residue analysis. Liquid chromatography–mass spectrometry (LC/MS)–grade formic acid, water, and acetonitrile were obtained from Fisher Scientific (Waltham, MA, USA). Anhydrous sodium acetate, magnesium sulfate, and the dispersive solid phase extraction (dSPE) sorbent SupelTM QuE Verde (Part No. 55442-U) were purchased from Sigma-Aldrich. Quick Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Dispersive Kits (Part No: 5982-5122) were purchased from Agilent (Santa Clara, CA, USA).

Pesticide extraction from leaf, flower petal, and nectar tissues. The pesticide residue analysis of leaves, flower petals, and nectar tissues used a modified QuEChERS method that was optimized for each plant tissue type. Five-hundred-milligram leaf samples were weighed into 7-mL screw-cap tubes. Before sample extraction, the internal standards were added at 100 ng/g fresh weight, followed by 1.5 mL MS-grade water and 10 to 15 metal homogenization beads in each tube. Samples were homogenized to a slurry using a Precellys Evolution homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) at 6000 rpm for five repetitions of 30-s cycles. Three milliliters MS-grade acetonitrile was added to the slurry and vortexed at 2500 rpm for 5 min; 800 ± 10 mg magnesium sulfate and 200 ± 5 mg sodium acetate were then

Table 2. Multiple Reaction Monitoring ion transitions for insecticides and their stable isotope-labeled internal standards.

Insecticide name	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)
Imidacloprid-olefin	252.0	205.10	11
		46.10	22
		81.10	11
Dinotefuran	203.1	129.10	–12
		114.10	–12
		73.10	–19
Dinotefuran- $^{13}\text{C}_5$	208.1	132.10	–12
		73.15	–21
		88.10	–16
Thiamethoxam	292.0	211.05	–12
		181.05	–23
		132.00	–20
Thiamethoxam- $^{13}\text{C}_4, ^{15}\text{N}$	297.0	216.00	–12
		186.00	–23
		136.95	–20
Clothianidin	250.0	169.05	–13
		113.10	–30
		132.00	–16
Clothianidin- $^{13}\text{C}_4, ^{15}\text{N}$	255.0	174.10	–12
		136.90	–16
		113.10	–28
Imidacloprid	256.0	175.10	–16
		209.00	–14
		210.00	–11
Imidacloprid- d_4	260.1	179.10	–19
		213.00	–15
		214.05	–10
Flupyradifurone	289.0	125.95	–20
		99.00	–48
		90.05	–40
Flupyradifurone- d_5	294.1	125.95	–26
		90.00	–39
		99.00	–45
Cyantraniliprole	475.0	285.90	–14
		443.85	–19
		112.00	–55
Cyantraniliprole- d_3	478.0	285.85	–15
		443.85	–20
		176.95	–45

added, and the samples were vortexed immediately at 1118 g_n for 5 min. Following centrifugation, aliquots of 1.6 mL supernatant were transferred to 500 mg \pm 5 mg SupelTM QuE Verde dSPE in 7-mL screw-cap tubes and shaken at 50 rpm for 10 min in a rotary shaker. Samples were then centrifuged at 1118 g_n for 5 min, and 800 μL of the supernatant was transferred to 2.0-mL glass vials for analysis on liquid chromatography–tandem mass spectrometry (LC-MS/MS).

For flower petal and nectar tissues, the following modifications were made to the extraction method to improve the sensitivity of analysis because of the relatively lower concentrations of pesticide residues in these tissues. Flower petal tissues (0.5 g) were spiked with SIL internal standards (120 ng/g), homogenized with 1.5 mL MS-grade water, and extracted with 3 mL acetonitrile. Magnesium sulfate and sodium acetate were added to extracts as in the procedure for leaves, and after centrifugation, 1.8 mL supernatant was transferred to 2-mL screw-cap tubes containing 250 mg QuEChERS Dispersive Kit (Agilent, Part 5982-5122) and shaken on rotary shaker for 10 min at 50 rpm. After centrifugation, 1 mL of the supernatant was transferred to 2.0-mL glass vials and dried under nitrogen gas stream at

30°C . Dried extracts were re-dissolved in 100 μL acetonitrile and transferred to polypropylene inserts for analysis on LC-MS/MS.

Nectar samples (0.4 mL) were spiked with SIL internal standards (25 ng/mL nectar), vortexed with 1.1 mL MS-grade water for 5 min at 1118 g_n and extracted with 2 mL acetonitrile by vortexing for 5 min at 2500 rpm. Magnesium sulfate and sodium acetate were added as in the procedure for leaves. After centrifugation, 1.8 mL of supernatant was transferred to 2-mL screw-cap tubes containing 250 mg QuEChERS Dispersive Kit (Agilent, Part 5982-5122). Extracts were then shaken, centrifuged, concentrated by evaporation under nitrogen stream, re-dissolved in 100 μL acetonitrile, and analyzed on LC-MS/MS as in the procedure for flower petal tissues.

Insecticide residue analysis on HPLC-MS/MS. Insecticide residues from leaf, flower petals, and nectar were analyzed on high-pressure liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS; Shimadzu LCMS-8040, Shimadzu Kyoto, Japan) following separation on a Kinetex XB-C18 column (2.6 μm 150 \times 3.0 mm; Phenomenex, Torrance, CA, USA). A solvent gradient was used with 0.1% formic acid in water (solvent A) and 0.1% formic acid in

acetonitrile (solvent B). The column was initially equilibrated at 5% solvent B at 0 min, increased to 10% B at 1.0 min, then to 95% B at 8.0 min, held at 95% B for 2 min until 10.1 min, and then decreased to 5% B at 10.1 min. The solvent flow rate was maintained at 0.4 mL/min and the injection volume for samples and standards was 2 μ L. Insecticides were ionized in an electrospray ionization source with the nebulizing gas at 3 L/min, drying gas at 12 L/min, heat block at 400 °C, desolvation line at 250 °C, and cone voltage set to 4.5 kV. Ions were analyzed in the mass spectrometer with the Multiple Reaction Monitoring ion transitions (Table 2).

Insecticide residues were quantified by normalizing with the SIL internal standards. For this, samples and external calibration standards were prepared with the same concentration of the internal standards, and the normalized peak areas were used in the calibration curve. By normalizing insecticide residues with the internal standards for each insecticide, we were able to accurately quantify the residue concentrations by controlling for all losses during the sample extraction, cleanup, and instrument analysis (via matrix effects in electrospray ionization). The external calibration curves ranged from 0.78 ng/mL to 800 ng/mL for the nonlabeled insecticide residue concentrations and were prepared by serial dilution of the 800 ng/mL nonlabeled insecticide standard mixture in acetonitrile containing the internal standard.

Statistical analyses. Nectar residues were normalized to average Brix concentration within each experiment for an accurate within-experiment comparison of nectar residues. Normalizing ppb in a sample was calculated by multiplying the ppb of a sample by the quotient of the measured Brix in a sample divided by the average Brix across the experiment.

Residue data from nectar, petals, and leaves were square-root transformed to normalize skewed distributions in the data set. Imidacloprid plus its olefin metabolite were statistically analyzed together; the same process was applied to thiamethoxam and its metabolite clothianidin. All means and standard errors reported are from the pretransformed data. Data obtained from CA and NJ experiments were analyzed separately because of different methods used in plant production/maintenance and nectar extraction, as well as length of experiments and different sample collection time-points. The data from NJ were combined due to identical methods implemented in 2018 and 2019 experiments. All statistical analyses were performed in JMP 16Pro (SAS Inc.).

Mixed model effects test with repeated measures and post hoc Tukey's tests. A mixed model effect test with repeated measures was implemented to test if the independent variables had statistically significant effects on the dependent variable. The dependent variable was the transformed residue concentrations whereas the independent variables (model effects) were Application Method, Application Rate, Time (weeks after treatment), Application Method \times Rate, Application Method \times Time, Time \times Rate, and Application Method \times Rate \times Time. An effect or combination of effects was considered to have a statistically significant effect on residue concentration (in ppb) when $P \leq 0.05$.

Tukey's tests were performed to explore the differences among residues as influenced by the independent variables. Results from Tukey's tests were considered statistically significant when $P \leq 0.05$.

Results

In both locations, Application Method (drench and foliar) had a significant effect on

the residues of all insecticides in all tissues, except for cyantraniliprole residues in the petals from the CA experiment and flupyradifurone residues in the leaves from the NJ experiment (Tables 3 and 4). Application rate (high and low) had a significant effect on the residues of all actives in all plant tissues in the CA experiment (Table 3). Rate had a significant effect on most insecticides in the NJ experiment except for dinotefuran residues in the petals and nectar (Table 4). Time (2, 4, 6, and 8 weeks after treatment in the CA experiment and 2, 6, and 10 weeks after treatment in the NJ experiment) had a significant effect in all cases except for cyantraniliprole residues found in the petals of the CA experiment and imidacloprid residues found in the nectar of the NJ experiment (Tables 3 and 4). However, statistical significance of interaction effects (Application Method \times Rate, Application Method \times Time, Rate \times Time, and Application Method \times Rate \times Time) was less consistent (Tables 3 and 4).

Generally, the highest concentrations of residues, regardless of active ingredient were found in the leaves and the lowest concentrations in nectar in both locations. Nectar concentrations ranged from 0.19 ± 0.03 ppb (low rate of cyantraniliprole applied as a foliar spray, at 10 weeks after treatment in NJ) to $42,352 \pm 1506$ ppb in leaves (high rate of dinotefuran applied as a drench, at 2 weeks after treatment in CA) (Tables 5–14). Drench applications typically resulted in higher residues than foliar applications, and samples from plants treated with the high application rates contained higher residues than the low rates, with varying statistical significance and some exceptions (Tables 5–14).

Dinotefuran residues in both plant tissues and nectar declined over time in both locations regardless of rate or application method

Table 3. *F* ratios of two-way analysis of variance with repeated measurements of effects of Application Method and dose on the concentration of five different active ingredients (imidacloprid, flupyradifurone, dinotefuran, thiamethoxam, and cyantraniliprole) on leaves, petals, and nectar of snapdragon 'Sonnet White' at 2, 4, 6, and 8 weeks after treatment (WAT) in California.

		Imidacloprid <i>F</i>	Flupyradifurone <i>F</i>	Dinotefuran <i>F</i>	Thiamethoxam <i>F</i>	Cyantraniliprole <i>F</i>
Leaves	Application method	1622.5***	44.5***	643.1***	627.9***	133.6***
	Dose	195.8***	75.9***	67.4***	141.9***	190.1***
	Time	75.7***	78.7***	293.0***	240.5***	7.9**
	Application method \times Dose	128.9***	0.05	29.4***	20.4***	125.2***
	Application method \times Time	14.6***	34.5***	92.8***	28.5***	5.8**
	Time \times Dose	9.3***	4.8**	9.6***	19.1***	4.7**
	Time \times Application method \times Dose	3.8*	1.9	8.4**	6.7**	3.1*
Petals	Application method	522.13***	12.1**	85.14**	3.03*	1.15
	Dose	66.76***	29.56***	7.45*	8.98**	9.34**
	Time	14.01***	33.40***	34.74***	17.56***	5.97
	Application method \times Dose	30.89**	7.55*	1.90	0.81	2.18
	Application method \times Time	5.72**	25.04***	15.89***	0.71	5.48*
	Time \times Dose	2.33*	10.34***	1.36	2.44	1.48
	Time \times Application method \times Dose	5.50**	14.99***	0.01	0.08	1.31
Nectar	Application method	474.85***	19.95***	303.93***	587.75***	322.23***
	Dose	41.45***	51.70***	21.80***	53.17**	52.32***
	Time	24.45***	71.23***	113.65***	76.35***	15.02***
	Application method \times Dose	24.95***	5.24*	10.49*	21.51**	3.36*
	Application method \times Time	26.51***	3.20*	61.26***	31.05***	1.14
	Time \times Dose	3.39**	6.13**	4.94**	4.54**	5.91*
	Time \times Application method \times Dose	4.20*	0.43	1.21	0.54	2.94*

There were two levels of Application method (drench and foliar) and two levels of doses (high and low). Leaves, petals, and nectar were collected at four different times (2, 4, 6, and 8 WAT).

*, **, *** indicate significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.0001$, respectively.

Table 4. *F* ratios of two-way analysis of variance with repeated measurements of effects of Application Method and dose on the concentration of five different active ingredients (imidacloprid, flupyradifurone, dinotefuran, thiamethoxam, and cyantraniliprole) on leaves, petals, and nectar of snapdragon ‘Sonnet Yellow’ at 2, 6, and 10 weeks after treatment (WAT) in New Jersey.

		Imidacloprid	Flupyradifurone	Dinotefuran	Thiamethoxam	Cyantraniliprole
		<i>F</i>	<i>F</i>	<i>F</i>	<i>F</i>	<i>F</i>
Leaves	Application method	192.8008***	0.0002	370***	365.6472***	61.3682***
	Dose	16.6921***	21.9623***	11.173**	53.07***	81.906***
	Time	25.5047***	51.2833***	16.688***	47.1763***	36.153***
	Application method × Dose	5.1911*	0.0372	4.205*	13.1021**	9.2116**
	Time × Application method	2.6207	25.4685***	4.742*	11.25***	16.8369***
	Time × Dose	1.1798	0.6926	0.179	3.7*	12.1839***
	Time × Application method × Dose	0.1842	0.0154	0.051	0.6178	12.1237***
Petals	Application method	95.531***	14.4807**	135.595***	175.94***	30.6617***
	Dose	13.2315**	19.747***	2.897	33.8472***	12.9361**
	Time	3.2765*	13.585***	12.963***	11.9561***	6.6079**
	Application method × Dose	6.6977*	0.1986	1.328	24.7815***	0.0001
	Time × Application method	0.5023	2.4869	5.58**	9.6141**	3.7741*
	Time × Dose	1.6282	2.3832	0.44	1.2451	0.1003
	Time × Application method × Dose	0.4532	1.1726	0.519	0.7326	0.0571
Nectar	Application method	136.56***	26.5875***	183.65791***	152.3045***	30.2032***
	Dose	15.857**	33.9755***	3.5072	22.447***	18.2757***
	Time	2.4376	22.3979***	18.175***	7.471**	13.1519***
	Application method × Dose	1.506**	4.6264*	1.434	8.077**	0.1224
	Time × Application method	1.605	3.7949*	9.222**	3.367*	1.9195
	Time × Dose	0.0973	0.68	0.2557	0.0839	2.405
	Time × Application method × Dose	0.741	0.1227	0.1919	0.1996	1.8522

There were two levels of Application method (drench and foliar) and two levels of doses (high and low). Leaves, petals, and nectar were collected at 2, 6, and 10 WAT.

*, **, *** indicate significant at Tukey’s $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.0001$, respectively.

(Tables 5 and 6), and this was significant in all cases except for nectar from CA treated with the low foliar rate ($df = 3$, $F = 0.0547$, $P = 0.0547$) and petals from NJ treated with the high drench rate ($df = 2$, $P = 0.089$).

Imidacloprid residues also decreased in the leaves and petals from both sites over time with varying statistical significance (Tables 7 and 8). In CA, imidacloprid residue concentrations from the high-rate drench treatments declined significantly over time from 447.5 to 80.8 ppb ($P = 0.0006$), but residues in plants treated with high-rate foliar applications remained low with a slight increase at week 8 (5.1 to 16.3 ppb, $P = 0.0006$) (Table 7). In NJ, there were several slight increases in concentrations over time, found mostly in the nectar, but none were found to be statistically significant (Table 8).

Thiamethoxam residues significantly declined in leaf tissue over time at both sites (Tables 9 and 10). Thiamethoxam levels in petals significantly decreased over time regardless of application method or application rate in the CA experiment (Table 9). In NJ, declines in residues over time were significant except in the nectar from the high-rate drench and the petals from foliar applications (Table 10). Also, residues from petals in CA tended to be lower than those from NJ for corresponding drench treatments (i.e., 43.5 ppb and 19.6 ppb in the CA experiment, but 489 ppb and 149.2 ppb in the NJ experiments at 2 weeks after treatment). However, the opposite was true for the foliar treatments (Tables 9 and 10). In NJ, thiamethoxam residues in nectar declined significantly after application throughout the sampled weeks in all except the high-rate drench treatment (means = 134.3, 90.22, 72.9; $df = 2$, $P = 0.1997$) (Table 10). Thiamethoxam residues from nectar in the CA experiment significantly

decreased over time in both drench treatments (Table 9), but residues increased by 8 weeks after treatment at both the high (week 6, 0.74 ppb; week 8, 2.47 ppb) and low application rates (week 6, 1.9 ppb; week 8, 2.19 ppb) in foliar applications, although increases were not statistically significant (Table 9).

Flupyradifurone residues decreased over time across tissues, nectar, rates, and application method in CA except for the low foliar and drench application rate residues found in nectar (Table 11). Flupyradifurone residues also similarly declined over time in NJ (Table 12), but the statistical significance was less consistent than in the CA experiment.

Cyantraniliprole residues generally decreased over time regardless of rate, tissue, nectar, or application method for both locations (Tables 13 and 14). However, cyantraniliprole residues in nectar from plants sprayed with the low application rate increased over time in the CA experiment (week 2 mean = 0.8 ± 0.2 , week 8 mean = 1.11 ± 0.5 ; $df = 3$, $P = 0.0773$), as did residues in the petals of the NJ experiment from plants treated with the high drench application rate (week 2 mean = 60.14 ± 14.45 , week 10 mean = 69.7 ± 36.3 ; $df = 2$, $P = 0.9504$).

Discussion and Conclusion

In this study, drench application resulted in greater concentrations of systemic insecticides than foliar application at the recommended label rates. Higher residue concentrations after drench applications vs. foliar sprays is consistent with other studies, such as in swamp milkweed nectar with dinotefuran, imidacloprid, and thiamethoxam (Cowles and Eitzer 2017). Residues in pollen and nectar have been

shown to be the primary exposure routes for honeybee colonies (Sanchez-Bayo and Goka 2014). Neonicotinoid concentrations of 1000 ppb and above have been found in the nectar and pollen of wildflowers and crops in Canada, Europe, and the United States (David et al. 2016; Long and Krupke 2016; Stewart et al. 2014; Tsvetkov et al. 2017; Woodcock et al. 2017). Pollen collected from 13 pollen traps from hives of honeybees foraging at three commercial ornamental plant nurseries in Connecticut indicated that honeybees foraged from diverse flower plants but were exposed to multiple active ingredients whose maximum ppb values ranged from 456 ppb (spiromesifen) down to 0.9 ppb (diazinon) (Stoner et al. 2019). Neonicotinoids have often been found in soils, aquatic environments, terrestrial ecotones, and crop systems in concentrations above the target pests’ LC₅₀ (Goulson 2013). Among 10 pesticides detected in 100 nectar samples taken from several agricultural settings in Pakistan, imidacloprid, thiamethoxam, and fipronil were the most abundant insecticides; comprising 8%, 6%, and 5% of the abundance of total pesticides detected, respectively (Pervez and Manzoor 2020).

Multiple factors influence pesticide residues collected by pollinators. A study in Italy noted increased insecticide concentrations in honeybees’ pollen loads when apple trees bloomed; pollen residues were similar inside and outside the orchards before and 2 weeks after bloom (Favaro et al. 2019). Beebread and pollen collected from five apiaries in China contained higher pesticide residue concentrations in spring and those of imidacloprid, thiamethoxam, fenpropathrin, bifenthrin, and chlorpyrifos commonly occurring within the beebread (Tong et al. 2018). Application method also can directly affect residue levels when using label rates. For example,

Table 5. Mean \pm SE of dinotefuran (ppb) in leaves, petals, and nectar extracted of *Antirrhinum majus* (snapdragon 'Sonnet White') treated with Safari 20SG. Samples were collected 2, 4, 6, and 8 weeks after treatment (WAT) from 15 replicate plants in California.

	Leaves						Petals						Nectar					
	Drench			Foliar			Drench			Foliar			Drench			Foliar		
	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value
2 WAT	42.352 \pm 1056Aa	18.294 \pm 1320Ab	<0.0001	2974 \pm 816Ac	2162 \pm 475Ac	<0.0001	666.3 \pm 215Aa	349.2 \pm 66Aa	<0.0001	48.5 \pm 8Ab	9.6 \pm 3Ab	0.0004	840.3 \pm 86Aa	389.4 \pm 81Ab	<0.0001	10.2 \pm 2.7Ac	3.0 \pm 0.8Bb	<0.0001
4 WAT	11,264 \pm 2026Ba	5,334 \pm 650Bb	<0.0001	991 \pm 180Bc	560 \pm 79Bc	<0.0001	310.6 \pm 109ABa	163.9 \pm 56Aab	<0.0001	14.1 \pm 10AB/c	3.2 \pm 0.8Bc	0.0032	330.0 \pm 92Ba	184.4 \pm 34Aa	<0.0001	10.2 \pm 4.1Bb	3.0 \pm 0.8Bb	0.0002
6 WAT	3068 \pm 679Ca	1740 \pm 471Ca	0.0006	285 \pm 63Cb	169 \pm 52BCb	0.0006	32.8 \pm 15Ba	11.3 \pm 5Bab	0.0006	1.7 \pm 0.0Bb	1.7 \pm 0.00Bb	0.0334	37.2 \pm 12Ca	11.2 \pm 6Bab	0.0025	0.5 \pm 0.08Cb	0.7 \pm 0.2Bb	0.0025
8 WAT	1322 \pm 152Ca	712 \pm 45Ca	0.0001	234 \pm 79Cb	82 \pm 40Cb	0.0001	16.1 \pm 1.4Ba	4.6 \pm 1.4Bb	0.0001	0.74 \pm 0.0Bb	1.7 \pm 0.00Bb	0.0137	10.8 \pm 6Ca	4.3 \pm 1.7Ba	0.0784	0.68 \pm 0.08Ca	2.0 \pm 1.2Ba	0.0784
P value	<0.0001	<0.0001	0.0002	0.0010	0.0002	0.0002	0.0059	0.0001	0.0059	0.0093	0.0046	0.0046	<0.0001	<0.0001	<0.0001	0.0547	0.0547	0.0547

Different **upper-case, bold letters** (within a column) indicate significant differences among dates (2, 4, 6, 8 WAT) within each treatment application (drench high, drench low, foliar high, or foliar low) as determined by Tukey's honestly significant difference (HSD). Different **lower-case, italicized letters** (across rows) indicate significant differences among treatment applications (drench high, drench low, foliar high, and foliar low), within each date (2, 4, 6, or 8 WAT) as determined by Tukey's HSD. All three matrices were analyzed separately.

Table 6. Mean \pm SE of dinotefuran (ppb) in leaves, petals, and nectar of *Antirrhinum majus* (snapdragon 'Sonnet Yellow') treated with Safari 20 SG. Samples were collected 2, 6, and 10 weeks after treatment (WAT) from 25 to 30 plants per block in 2018 and 2019 in New Jersey.

	Leaves						Petals						Nectar					
	Drench			Foliar			Drench			Foliar			Drench			Foliar		
	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value
2 WAT	20605 \pm 2617Aa	13975 \pm 2089Aa	<0.0001	1502 \pm 186.3Ab	871.6 \pm 116Ab	<0.0001	3777 \pm 1012Aa	3641 \pm 1271Aa	<0.0001	113.7 \pm 26.4Ab	70.5 \pm 8.5Ab	<0.0001	852 \pm 103Aa	562 \pm 117Aa	<0.0001	20 \pm 2.3Ab	12.6 \pm 2.6Ab	<0.0001
6 WAT	18645 \pm 2328ABa	11951 \pm 2414ABa	<0.0001	477.9 \pm 47.9Bb	263.2 \pm 47.2Bb	<0.0001	1789 \pm 526Aa	1351 \pm 554ABa	<0.0001	34.3 \pm 6.1Bb	22.2 \pm 8.3Bb	<0.0001	434 \pm 295ABa	312.5 \pm 64ABa	<0.0001	9.6 \pm 3.5Bb	4.5 \pm 1.5Bb	<0.0001
10 WAT	9883 \pm 3337Ba	6151 \pm 1831Ba	<0.0001	185.6 \pm 81.6Bb	185.6 \pm 81.6Bb	<0.0001	1606 \pm 555Aa	525.2 \pm 286Bab	<0.0001	16.95 \pm 5.1Bb	6.7 \pm 2.4Bb	<0.0001	231 \pm 107Ba	146 \pm 71Ba	0.0009	3.77 \pm 1.2Bb	2.43 \pm 1.1Bb	0.0009
P value	0.018	0.0386	<0.0001	<0.0001	<0.0001	<0.0001	0.089	0.009	0.0003	0.0003	<0.0001	0.0003	0.0092	0.0095	0.0011	0.0028	0.0028	0.0028

Different **upper-case, bold letters** (within a column) indicate significant differences among dates (2, 6, 10 WAT) within each treatment application (drench high, drench low, foliar high, or foliar low) as determined by Tukey's honestly significant difference (HSD). Different **lower-case, italicized letters** (across rows) indicate significant differences among treatment applications (drench high, drench low, foliar high, and foliar low), within each date (2, 6, or 10 WAT) as determined by Tukey's HSD. All three matrices were analyzed separately.

Table 7. Mean \pm SE of imidacloprid (ppb) plus its degradant olefin in leaves, petals, and nectar of *Antirrhinum majus* (snapdragon 'Sonnet White') treated with Marathon II + Altus. Samples were collected 2, 4, 6, and 8 weeks after treatment (WAT) from 15 replicate plants in California.

	Leaves						Petals						Nectar					
	Drench			Foliar			Drench			Foliar			Drench			Foliar		
	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value
2 WAT	15,161 \pm 807Aa	5008 \pm 613Ab	<0.0001	825 \pm 263Ac	335 \pm 74Ac	<0.0001	539.5 \pm 77ABa	322.8 \pm 57Aab	<0.0001	142.5 \pm 43ABc	38.5 \pm 7.5Ac	0.0003	447.5 \pm 79Aa	142.6 \pm 11.4Ab	<0.0001	5.1 \pm 0.7Bc	3.9 \pm 0.65Ac	<0.0001
4 WAT	10735 \pm 702Ba	3134 \pm 629Bb	<0.0001	219 \pm 19Bc	142.6 \pm 22Bc	<0.0001	746.3 \pm 108Aa	235 \pm 22ABb	<0.0001	31.0 \pm 0.00Bc	31.0 \pm 0.00Ac	<0.0001	182.3 \pm 22.4Bb	83.5 \pm 15.4ABb	<0.0001	2.1 \pm 0.4Bc	1.2 \pm 0.2Ac	<0.0001
6 WAT	5999 \pm 543Ca	2212 \pm 366Bb	<0.0001	99.9 \pm 14Bc	55.5 \pm 15Cc	<0.0001	352.3 \pm 8Ba	169 \pm 10Bb	<0.0001	31.0 \pm 0.00Bc	31.0 \pm 0.00Ac	<0.0001	89.5 \pm 18Ba	50.1 \pm 6Ba	<0.0077	1.7 \pm 0.2Bb	2.8 \pm 1.6Ab	<0.0077
8 WAT	4298 \pm 455Ca	1973 \pm 80Bb	<0.0001	40 \pm 3Bc	36.3 \pm 3Cc	<0.0001	364.9 \pm 66Ba	172.4 \pm 22Bb	<0.0001	31.0 \pm 0.00Bc	31.0 \pm 0.00Ac	<0.0001	80.8 \pm 5.2Ba	41.3 \pm 18Bab	0.07	16.3 \pm 6.9Ab	3.7 \pm 1.8Ab	0.07
P value	<0.0001	0.0088	0.0005	0.0007	0.0007	0.0007	0.0145	0.0311	0.0016	0.0016	0.4411	0.0006	0.0006	0.0204	0.0006	0.1496	0.1496	0.1496

Different **upper-case, bold letters** (within a column) indicate significant differences among dates (2, 4, 6, 8 WAT) in each treatment application (drench high, drench low, foliar high, or foliar low) as determined by Tukey's honestly significant difference (HSD). Different **lower-case, italicized letters** (across rows) indicate significant differences among treatment applications (drench high, drench low, foliar high, and foliar low), in each date (2, 4, 6, or 8 WAT) as determined by Tukey's HSD. All three matrices were analyzed separately.

Table 8. Mean \pm SE of imidacloprid (ppb) plus its degradant olefin in leaves, petals, and nectar of *Antirrhinum majus* (snapdragon 'Sonnet Yellow') treated with Marathon II+ Altus. Samples were collected 2, 6, and 10 weeks after treatment (WAT) from 25 to 30 plants per block in 2018 and 2019 in New Jersey.

	Leaves						Petals						Nectar					
	Drench			Foliar			Drench			Foliar			Drench			Foliar		
	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value
2 WAT	1477 \pm 311Aa	771 \pm 132Aab	0.0002	263.5 \pm 49Ab	467.2 \pm 140Ab	0.0002	180 \pm 42.5Aa	69.4 \pm 22.5Ab	0.0002	30 \pm 6.5Ab	24.4 \pm 16.7Ab	0.0002	35.8 \pm 12Aa	12.1 \pm 1.7Aab	0.0002	5.74 \pm 2.7Ab	4.75 \pm 3.3Ab	0.002
6 WAT	1164 \pm 200ABa	552.4 \pm 71.3Ab	<0.0001	33.5 \pm 3.7Bc	59.9 \pm 15Bc	<0.0001	136.4 \pm 35.3Aa	43.7 \pm 9Ab	<0.0001	24.4 \pm 3Ab	24.4 \pm 3Ab	0.0004	39.5 \pm 10.2Aa	18.7 \pm 3.4Aab	0.0004	4.7 \pm 3.3Ab	5.76 \pm 2.3Ab	0.0002
10 WAT	717.1 \pm 106.6Ba	500.2 \pm 123Aa	<0.0001	34.9 \pm 10.5Bb	37.5 \pm 13.6Bb	<0.0001	80.14 \pm 25.5Aa	56.5 \pm 23.4Aa	<0.0001	24.4 \pm 3Ab	24.4 \pm 3Ab	0.0296	22.5 \pm 4Aa	15.2 \pm 7.5Aab	0.0296	5.86 \pm 2.3Ab	4.8 \pm 2.0Ab	0.007
P value	0.0543	0.2327	<0.0001	0.0002	0.0002	<0.0001	0.1733	0.7307	0.1733	0.6918	1.000	1.000	0.3264	0.4886	0.3264	0.9091	0.9434	

Different **upper-case, bold letters** (within a column) indicate significant differences among dates (2, 6, 10 WAT) in each treatment application (drench high, drench low, foliar high, or foliar low) as determined by Tukey's honestly significant difference (HSD). Different *lower-case, italicized letters* (across rows) indicate significant differences among treatment applications (drench high, drench low, foliar high, and foliar low), within each date (2, 6, or 10 WAT) as determined by Tukey's HSD. All three matrices were analyzed separately.

Table 9. Mean \pm SE of thiamethoxam + clothianidin (ppb) in leaves, petals, and nectar of *Antirrhinum majus* (snapdragon bedding 'Sonnet White') treated with Flagship 25WG + Mainspring GNL (Syngenta Crop Protection LLC, Greensboro, NC, USA). Samples were collected 2, 4, 6, and 8 weeks after treatment (WAT) from 15 replicate plants in California.

	Leaves						Petals						Nectar					
	Drench			Foliar			Drench			Foliar			Drench			Foliar		
	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value
2 WAT	14133.6 \pm 1126Aa	4667.3 \pm 14Ab	<0.0001	1965.4 \pm 193Ac	774.0 \pm 166Ad	<0.0001	43.5 \pm 14Aa	19.6 \pm 4.5Aa	0.1066	27.9 \pm 8.4Aa	13.6 \pm 4.3Aa	0.1066	97.39 \pm 7.6Aa	51.26 \pm 1.99Ab	0.1066	10.05 \pm 0.74Ac	3.73 \pm 1.32Ad	<0.0001
4 WAT	4749.6 \pm 953Ba	2434.6 \pm 383Bab	<0.0001	80.9 \pm 11Bc	946.2 \pm 222Bb	<0.0001	16.3 \pm 7ABa	7.4 \pm 0.5Ba	0.0623	10.8 \pm 4ABa	6.8 \pm 0.00Ba	0.0623	49.16 \pm 6.08Ba	24.19 \pm 4.15Bb	0.0623	3.10 \pm 0.86Bc	0.5 \pm 0.06Bc	<0.0001
6 WAT	1855 \pm 219Ca	554.2 \pm 27Cb	<0.0001	105.3 \pm 72Cc	142.2 \pm 4Bc	<0.0001	10.9 \pm 2ABa	6.8 \pm 0.00Ba	0.05	6.8 \pm 0.00Ba	6.8 \pm 0.00Ba	0.05	27.9 \pm 5.48BCa	12.38 \pm 2.4Cb	0.05	0.74 \pm 0.7Bc	1.9 \pm 0.8ABc	<0.0041
8 WAT	888.3 \pm 70Ca	515.9 \pm 58Cb	<0.0001	10.1 \pm 0.3Cc	11.7 \pm 1Bc	<0.0001	6.8 \pm 0.00Ba	6.8 \pm 0.00Ba	—	6.8 \pm 0.00Ba	6.8 \pm 0.00Ba	—	14.80 \pm 2.41Ca	6.9 \pm 0.77Cab	—	2.47 \pm 1.6Bb	2.19 \pm 0.4ABb	<0.4712
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0248	0.0001	0.0207	0.0176	0.0176	<0.0001	<0.0001	<0.0001	<0.0001	0.2315	0.2609	

Different **upper-case, bold letters** (within a column) indicate significant differences among dates (2, 4, 6, 8 WAT) within each treatment application (drench high, drench low, foliar high, or foliar low) as determined by Tukey's honestly significant difference (HSD). Different *lower-case, italicized letters* (across rows) indicate significant differences among treatment applications (drench high, drench low, foliar high, and foliar low), within each date (2, 4, 6, or 8 WAT) as determined by Tukey's HSD. All matrices were analyzed separately.

Table 10. Mean \pm SE of thiamethoxam plus its degradant clothianidin (ppb) in leaves, petals, and nectar of *Antirrhinum majus* (snapdragon 'Cultivar') treated with Flagship 25WG + Mainspring GNL (Syngenta Crop Protection LLC, Greensboro, NC, USA). Samples were collected 2, 6, and 10 weeks after treatment (WAT) from 25 to 30 plants per block in 2018 and 2019 in New Jersey.

	Leaves						Petals						Nectar					
	Drench			Foliar			Drench			Foliar			Drench			Foliar		
	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value	High	Low	P value
2 WAT	14901 \pm 3856Aa	5387 \pm 951.2Ab	<0.0001	1067 \pm 156.6Ac	229.8 \pm 72Ac	<0.0001	489 \pm 103Aa	149.2 \pm 16.6Ab	<0.0001	14.11 \pm 2.39Ac	5.38 \pm 0.84Ac	<0.0001	134.3 \pm 18.5Aa	51.8 \pm 5.73Ab	<0.0001	8.04 \pm 2.0Ac	1.48 \pm 0.4Ac	<0.0001
6 WAT	6332 \pm 823.1Ba	2405 \pm 353Bb	<0.0001	157.1 \pm 30Bc	37 \pm 17.6Bc	<0.0001	254.5 \pm 107ABa	86.67 \pm 18.6ABb	<0.0001	9.5 \pm 1.8Ac	4.45 \pm 1.1Ac	<0.0001	90.22 \pm 17Aa	41.2 \pm 4.9Aa	<0.0001	3.71 \pm 0.9ABb	0.42 \pm 0.14Bb	<0.0001
10 WAT	3162.3 \pm 371.3Ba	1324.4 \pm 221Bb	<0.0001	136.5 \pm 56.4Bc	13.6 \pm 9.8Bc	<0.0001	193.1 \pm 72Ba	47.4 \pm 9.9Ab	<0.0001	8.32 \pm 3.45Ac	4.4 \pm 1.11Ac	<0.0001	72.9 \pm 40Aa	15.87 \pm 4Bab	<0.0001	2.92 \pm 1.5Bb	0.2 \pm 0.03Bb	0.0003
P value	0.0037	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	0.0415	0.0019	0.0019	0.1943	0.7056	0.1997	0.0007	0.0007	0.0396	0.0073	0.0023	

Different **upper-case, bold letters** (within a column) indicate significant differences among dates (2, 6, and 10 WAT) within each treatment application (drench high, drench low, foliar high, or foliar low) as determined by Tukey's honestly significant difference (HSD). Different *lower-case, italicized letters* (across rows) indicate significant differences among treatment applications (drench high, drench low, foliar high, and foliar low), within each date (2, 6, or 10 WAT) as determined by Tukey's HSD. All matrices were analyzed separately.

Table 11. Mean \pm SE of flupyradifurone (ppb) in leaves, petals, and nectar of *Antirrhinum majus* (snapdragon ‘Sonnet White’) treated with Marathon II + Altus. Samples were collected 2, 4, 6, and 8 weeks after treatment (WAT) from 15 replicate plants in California.

WAT	Leaves			Petals			Nectar		
	High	Low	P value	High	Low	P value	High	Low	P value
2 WAT	1873.3 \pm 31Aa	733.7 \pm 40Ac	3332.9 \pm 415Ab	91.0 \pm 14Bb	78.5 \pm 12Ab	0.0001	166.6 \pm 68Ab	17.5 \pm 3.78Abc	0.0059
4 WAT	1746.5 \pm 79Aa	600.1 \pm 85ABb	1287.6 \pm 309Bcb	149.5 \pm 10Aa	49.3 \pm 17ABa	0.0051	167.3 \pm 63Aa	3.76 \pm 1.25Bb	0.0108
6 WAT	1037.4 \pm 25Ba	498.3 \pm 67ABb	541.2 \pm 111BCb	71.6 \pm 11Bb	28.4 \pm 1ABb	0.0014	8.4 \pm 0.0Bc	1.4 \pm 0.5Bb	0.0372
8 WAT	827.9 \pm 103Ba	392.8 \pm 21Bb	315.3 \pm 72Cb	92.3 \pm 15Ba	38.9 \pm 4Bb	0.0086	8.4 \pm 0.0Bc	1.9 \pm 0.5Bb	0.3667
P value	<0.0001	0.0192	0.0002	0.0098	0.0435	0.0002	0.0029	0.0002	0.0668

Different **upper-case, bold letters** (within a column) indicate significant differences among dates (2, 4, 6, 8 WAT) within each treatment application (drench high, drench low, foliar high, or foliar low) as determined by Tukey’s honestly significant difference (HSD). Different **lower-case, italicized letters** (across rows) indicate significant differences among treatment applications (drench high, drench low, foliar high, and foliar low), within each date (2, 4, 6, or 8 WAT) as determined by Tukey’s HSD. All three matrices were analyzed separately.

Table 12. Mean \pm SE of flupyradifurone (ppb) in leaves, petals, and nectar of *Antirrhinum majus* (snapdragon ‘Sonnet Yellow’) treated with Marathon II + Altus. Samples were collected 2, 6, and 10 weeks after treatment (WAT) from 25 to 30 plants per block in 2018 and 2019 in New Jersey.

WAT	Leaves			Petals			Nectar		
	High	Low	P value	High	Low	P value	High	Low	P value
2 WAT	1859.2 \pm 296Aa	964.3 \pm 132Aab	4663.3 \pm 1498.2Ab	169.8 \pm 43.9Aab	81.24 \pm 27.8Aab	0.0021	28.3 \pm 5.8Aa	10.9 \pm 1.1Ab	0.0179
6 WAT	1381 \pm 233.3Aa	691.5 \pm 65.74Ab	710.14 \pm 150.2Bb	44.78 \pm 14.23Aab	33.27 \pm 10.53Bb	<0.0001	23.64 \pm 4.6ABa	11.3 \pm 1.4Ab	0.0037
10 WAT	1068.5 \pm 200.4Aa	664.2 \pm 121.7Aab	471.7 \pm 78Bbc	78.65 \pm 20.9Aa	57.12 \pm 22.85Aab	0.0004	13.68 \pm 5.25Bb	5.54 \pm 1.4Bb	0.0065
P value	0.1151	0.1285	<0.0001	0.2332	0.5078	0.0003	0.0411	0.0063	0.0112

Different **upper-case, bold letters** (within a column) indicate significant differences among dates (2, 6, 10 WAT) within each treatment application (drench high, drench low, foliar high, or foliar low) as determined by Tukey’s honestly significant difference (HSD). Different **lower-case, italicized letters** (across rows) indicate significant differences among treatment applications (drench high, drench low, foliar high, and foliar low), within each date (2, 6, or 10 WAT) as determined by Tukey’s HSD. All matrices were analyzed separately.

higher mean concentrations of dinotefuran, thiamethoxam, and imidacloprid found in swamp milkweed nectar when applied as a drench, compared with a foliar spray, up to 2 weeks before bloom (Cowles and Eitzer 2017). Imidacloprid formulated as granular product applied at 1x and 2x label rates to tropical milkweed (*Asclepias curassavica*) grown in 3-gal pots (300 and 600 mg a.i. per pot) yielded very large concentrations in the flowers 3 weeks after treatment (6030 and 10,400 ppb, respectively (Krischik et al. 2015). Note, however, the label rate for the granular imidacloprid formulation results in higher active ingredient amounts being applied than comparable liquid imidacloprid drench formulations (5.6 and 11.3 mg a.i. per 1-gal pot). Conversely, maize and oil rape-seed seeds treated with thiamethoxam showed very low residues in honey and bee bread, with experimental hives showing no significant differences in colony health when analyzed against the control group (Pilling et al. 2013).

Application to open flowers can deposit insecticides directly onto pollen such that concentrations in both leaves and pollen are similar (Lentola et al. 2017). Residues of cyantraniliprole and its metabolites varied depending on tomato tissue type, tissue age, time after treatment, and ripening stage of fruits (Huynh et al. 2021). Additional perturbations, such as shifts in climate and climate extremes, have been shown to affect the level of insecticide, herbicide, and fungicide expenditure when treating potato, soybean, corn, and wheat crops (Rhodes and McCarl 2020), affecting pollinator exposure.

In this study, residues within leaves, flower petals, and nectar showed similar trends between the two locations, but residue variability did exist. For example, imidacloprid concentrations in nectar were highly variable after drench applications between the two locations. Residues in the CA experiment averaged 447.5 ppb with the high rate and 79.38 ppb with the low rate in nectar 2 weeks after treatment. Eight weeks after treatment, the mean residues for high and low drench application rates declined to 80.8 ppb and 41.3 ppb, respectively. In the NJ experiment, nectar residues were lower than in the CA experiment, at 35.8 and 12.1 ppb with the high and low rates 2 weeks after treatment, respectively, whereas concentrations declined to 22.5 and 15.2 ppb 10 weeks after treatment. Factors contributing to these differences may be pot size and shape, soilless media composition, growing temperature, irrigation, etc. The CA and NJ sites used different-sized containers in this study: 0.656l deepots in CA and 6.435l classic pots in NJ. Moisture in soil can affect the half-life of acetamiprid (Gupta and Gajbihiye 2007) and thiamethoxam (Gupta et al. 2008), with drier conditions increasing the half-life dramatically. In addition, the nectar extraction process was different between the sites, with NJ pipetting nectar directly from the base of the snapdragon flower, a process that essentially mimics how a pollinator would collect nectar, whereas the CA location harvested flowers and collected nectar in the laboratory, which could concentrate residues. This direct

Table 13. Mean \pm SE of cyantraniliprole (ppb) in leaves, petals, and nectar of *Antirrhinum majus* (snapdragon ‘Sonnet White’) treated with Flagship 25WG + Mainspring GNL. Samples were collected 2, 4, 6, and 8 weeks after treatment (WAT) from 15 replicate plants in California.

WAT	Leaves				Petals				Nectar				
	Drench		Foliar		Drench		Foliar		Drench		Foliar		
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	
2 WAT	1283.2 ± 61Ab	503.4 ± 29Ab	23795.3 ± 3288Aa	1415.6 ± 275Ab	<0.0001	59.2 ± 20Aa	25.7 ± 10Aa	1035.4 ± 559Aa	167.0 ± 81Aa	0.0895	14.88 ± 0.8Aa	7.89 ± 0.35Ab	4.4 ± 0.73Ac
4 WAT	1378.7 ± 261Ab	620.0 ± 73Ab	22271.9 ± 4449Aa	316.5 ± 81 Bb	<0.0001	85.7 ± 14Aa	31.9 ± 6Aa	355.6 ± 268Aa	6.6 ± 4ABa	0.0857	10.6 ± 0.9ABa	5.4 ± 0.55Bb	2.98 ± 0.7ABb
6 WAT	1163.4 ± 265Ab	541.1 ± 96Ab	14430.4 ± 1164ABa	790.0 ± 247Bb	<0.0001	49.5 ± 6Aa	24.7 ± 3Aab	12.2 ± 5Bbc	2.4 ± 0.00Bc	0.0007	8.37 ± 1.5ABa	5.2 ± 0.57Ba	0.33 ± 0.1BCb
8 WAT	826.3 ± 38Aab	543.8 ± 30Aab	6686.3 ± 3209Ba	362.3 ± 162Bb	0.0271	53.9 ± 4.2Aa	18.9 ± 0.4Ab	2.4 ± 0.00Bc	2.4 ± 0.00Bc	<0.0001	8.06 ± 1.72Ba	3.6 ± 0.27Bab	0.92 ± 0.6Ccb
P value	0.2659	0.640	0.0227	0.0263		0.3066	0.6122	0.0554	0.0176		0.0343	0.0014	0.0773

Different **upper-case, bold letters** (within a column) indicate significant differences among dates (2, 4, 6, 8 WAT) within each treatment application (drench high, drench low, foliar high, or foliar low) as determined by Tukey’s honestly significant difference (HSD). Different *lower-case, italicized letters* (across rows) indicate significant differences among treatment applications (drench high, drench low, foliar high, and foliar low), within each date (2, 4, 6, or 8 WAT) as determined by Tukey’s HSD. All matrices were analyzed separately.

Table 14. Mean \pm SE of cyantraniliprole (ppb) in leaves, petals, and nectar of *Antirrhinum majus* (Snapdragon ‘Sonnet Yellow’) treated with Flagship 25WG + Mainspring GNL. Samples were collected 2, 6, and 10 weeks after treatment (WAT) from 25 to 30 plants per block in 2018 and 2019 in New Jersey.

WAT	Leaves				Petals				Nectar						
	Drench		Foliar		Drench		Foliar		Drench		Foliar				
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low			
2 WAT	1067.5 ± 201.6Ab	551.9 ± 91.4Abc	2505.3 ± 681.5Aa	77.9 ± 16.75Ac	<0.0001	60.14 ± 14.45Aa	32.48 ± 6.4Aab	97.5 ± 40.5Aab	10.9 ± 8.7Ab	0.0176	13.42 ± 3Aa	5.87 ± 0.93Aab	19.58 ± 6.36Aa	1.27 ± 0.21Ab	0.0031
6 WAT	900.8 ± 153Aa	386.5 ± 68.2Ab	140.8 ± 38.2Bc	6.1 ± 5Bd	<0.0001	58.6 ± 22Aa	35.7 ± 15.9Aa	3.28 ± 1Bb	1.7 ± 0.33Ab	0.0005	9.71 ± 2Aa	3.76 ± 1.45ABb	0.56 ± 0.057Bc	0.202 ± 0.03Bc	<0.0001
10 WAT	728.5 ± 106.5Aa	348.8 ± 68.5Ab	49.6 ± 6.25Bc	1.11 ± 0.05Bd	<0.0001	69.7 ± 36.3Aa	17.9 ± 1.97Aab	1.84 ± 0.3Bb	1.7 ± 0.33Ab	0.0003	7.25 ± 2.46Aa	2.26 ± 0.38Bb	0.47 ± 0.1B/c	0.19 ± 0.03Bb	<0.0001
P value	0.4234	0.1899	<0.0001	<0.0001	0.3693	0.9504	0.3693	0.0016	0.2627	0.0004	0.2291	0.0119	0.0004	0.019	0.0001

Different **upper-case, bold letters** (within a column) indicate significant differences among dates (2, 6, 10 WAT) within each treatment application (drench high, drench low, foliar high, or foliar low) as determined by Tukey’s honestly significant difference (HSD). Different *lower-case, italicized letters* (across rows) indicate significant differences among treatment applications (drench high, drench low, foliar high, and foliar low), within each date (2, 6, or 10 WAT) as determined by Tukey’s HSD.

pipetting process, in theory, should be more representative of bee nectar collection and exposure and is recommended for future studies.

The Environmental Protection Agency has established concentration levels of concern for each active ingredient within nectar above which there is greater risk to negatively affect honeybee health. The level of concern for ingestion is 25 ppb for imidacloprid, 55 ppb for cyantraniliprole, 35 ppb for thiamethoxam, 0.4 ppb for dinotefuran, and 10,000 ppb for flupyradifurone. Results of this study support current label advisories against drench applications of tested systemic neonicotinoids made pre-bloom to minimize exposure and risk. For flupyradifurone, residues in leaves, flower petals, or nectar did not exceed the level of concern, so when used alone, this active ingredient may be a candidate for applications to manage pests with limited impact on bee populations. Cyantraniliprole residues in nectar never exceeded 20 ppb regardless of application method or rate and may also provide an additional option for management of pests with minimal to no risk of impact on bee populations. In honeybees, imidacloprid had no adverse effects on worker mortality or overwintering deaths after chronic ingestion exposure at typical concentrations that honeybees would encounter in the wild (2–20 ppb) (Faucon et al. 2005; Johnson et al. 2010). The mean nectar residues of the foliar application of imidacloprid in this study fell within this 2 to 20 ppb range, whereas drench applications were often higher than 20 ppb. This suggests foliar applications of imidacloprid as a safer avenue than the drench in terms of pollinator conservation.

The negative effects certain concentrations of systemic insecticides have on honeybee colony health can be increased despite using lower rates when other variables are introduced, such as a concentration thought of as relatively safe to pollinators can be harmful when another stressor is introduced (Alaux et al. 2010). This study compared the additive effect the parasitic microsporidian *Nosema ceranae* had on colony health and mortality when introduced to colonies being exposed to 0.7, 7, and 70 ppb of imidacloprid via ingestion (Alaux et al. 2010). Over a 10-day period, the imidacloprid + *Nosema* treatment group showed higher bee mortality at all three concentrations than the imidacloprid or *Nosema* treatment groups individually and the control (Alaux et al. 2010). In this current study, nectar residues fell within, and in certain cases well over, the concentrations used in Alaux et al. (2010), suggesting the risk of applying imidacloprid even at lower rates in areas where honeybee colonies are susceptible to parasites.

Thoughtful experimental designs involving field-like conditions are key to understanding how insecticide residues truly move throughout the systems in which they are applied, creating realistic exposure values to nontarget organisms such as pollinators and target pests alike. For instance, a 2018 study measuring the DT50, the time required for the quantity of a compound to degrade by half, showed the lowest DT50 values (measured in days) were found in soil cores from

the field compared with soil cores under regimented light and moisture conditions in the laboratory (Hilton et al. 2018). Spatiotemporal modeling has shown that bioaccumulation of pesticides is higher in dry and arid regions, compared with colder and more humid conditions (Li 2022a). Such studies could potentially be very valuable to understand behavior of residues in established horticultural plants. Multiyear studies of systemic insecticide residues should be considered in the environmental horticulture industry, as herbaceous and especially woody perennials form a large part of the industry and provide a multiyear resource for the local pollinators. Factors such as solubility can affect the plant's ability to take up and metabolize systemic insecticides, as shown with dinotefuran and imidacloprid drenches on *Ilex × attenuata*, and *Clethra alnifolia* in Mach et al. (2017). Hilton et al. (2018) also used different soil types as an independent variable taken from different parts of Europe. More studies such as this could be especially valuable, given the variance in regional geology and climate. Exposure assessments by simulating residue levels in pollen and nectar could potentially be tools in mitigating the negative impacts insecticides can have on honeybees. Inputting physiochemical properties of an insecticide chemistry into a model accounting for factors such as application, geographic, environmental, and plant physiological variability can show the importance that these factors can have on nectar and pollen residue when assessing risk to honeybees (Li 2022b).

Foliar applications of systemic insecticides in some cases can be more effective than drench applications. For example, in a study of 10 translaminar and systemic pesticides to determine efficacy for the sweetpotato whitefly (*Bemisia tabaci*) infesting poinsettia, foliar-applied imidacloprid, dinotefuran, flupyradifurone, and cyantraniliprole was more effective in reducing nymph density compared with drenches up to 56 d after treatment in some cases (Gill and Chong 2021). Where drench applications may not be preferred, foliar application may be a viable methodology to manage certain pests effectively while lower pollinator exposure in pollen and nectar. In addition, using insecticides selectively in combination with insect predators that target the same pest as the insecticide can sometimes provide high efficacy (Torres and de F. Bueno 2018), these integrated strategies also have potential to reduce pollinator exposure while still maintaining acceptable levels of pest management.

References Cited

- Alaux C, Brunet JL, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, Brillard J, Baldy A, Belzunces LP, Le Conte Y. 2010. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environ Microbiol*. 12(3):774–782. <https://doi.org/10.1111/j.1462-2920.2009.02123.x>.
- Baron GL, Raine NE, Brown MJF. 2017. General and species-specific impacts of a neonicotinoid insecticide on the ovary development and feeding of wild bumblebee queens. *Proc Biol Sci*. 284. <https://doi.org/10.1098/rspb.2017.0123>.
- Byrne FJ, Toscano NC. 2006. Uptake and persistence of imidacloprid in grapevines treated by chemigation. *Crop Prot*. 25:831–834. <https://doi.org/10.1016/j.cropro.2005.11.004>.
- Castle SJ, Byrne FJ, Bi JL, Toscano NC. 2005. Spatial and temporal distribution of imidacloprid and thiamethoxam in citrus and impact on *Homalodisca coagulata* (Say) populations. *Pest Manag Sci*. 61:75–84. <https://doi.org/10.1002/ps.949>.
- Cowles RS, Eitzer BD. 2017. Residues of neonicotinoid insecticides in pollen and nectar from model plants. *J Environ*. 35(1):24–34. <https://doi.org/10.24266/0738-2898-35.1.24>.
- David A, Botías C, Abdul-Sada A, Nicholls E, Rotheray EL, Hill EM, Goulson D. 2016. Widespread contamination of wildflower and bee-collected pollen with complex mixtures of neonicotinoids and fungicides commonly applied to crops. *Environ Int*. 88:169–178. <https://doi.org/10.1016/j.envint.2015.12.011>.
- Dively GP, Embrey MS, Kamel A, Hawthorne DJ, Pettis JS. 2015. Assessment of chronic sublethal effects of imidacloprid on honeybee colony health. *PLoS One*. 10(3). <https://doi.org/10.1371/journal.pone.0181297>.
- Erickson E, Adam S, Russo L, Wojcik V, Patch HM, Grozinger CM. 2020. More than meets the eye? The role of annual ornamental flowers in supporting pollinators. *Environ Entomol*. 49(1):178–188. <https://doi.org/10.1093/ee/nvz133>.
- Faucon J-P, Aurières C, Drainudel P, Mathieu L, Ribiere M, Martel AC, Zeggane S, Chauzat M-P, Aubert MFA. 2005. Experimental study on the toxicity of imidacloprid given in syrup to honeybee (*Apis mellifera*) colonies. *Pest Manag Sci*. 61:111–125. <https://doi.org/10.1002/ps.957>.
- Favaro R, Bauer LM, Rossi M, D'Ambrosio L, Bucher E, Angeli S. 2019. Botanical origin of pesticide residues in pollen loads collected by honeybees during and after apple bloom. *Front Physiol*. 10. <https://doi.org/10.3389/fphys.2019.01069>.
- Garbuzov M, Ratnieks FLW. 2014. Quantifying variation among garden plants in attractiveness to bees and other flower-visiting insects. *Funct Ecol*. 28(2):364–374. <https://doi.org/10.1111/1365-2435.12178>.
- Gill GS, Chong JH. 2021. Efficacy of selected insecticides as replacement for neonicotinoids in managing sweetpotato whitefly on poinsettia. *HortTechnology*. 31(6):745–752. <https://doi.org/10.1111/1365-2435.12178>.
- Goulson D. 2013. An overview of the environmental risks posed by neonicotinoid insecticides. *J Appl Ecol*. 50:977–987. <https://doi.org/10.1111/1365-2664.12111>.
- Gupta S, Gajbhiye VT. 2007. Persistence of acetamiprid in soil. *Bull Environ Contam Toxicol*. 78:349–352. <https://doi.org/10.1007/s00128-007-9097-7>.
- Gupta S, Gajbhiye VT, Gupta RK. 2008. Soil dissipation and leaching behavior of a neonicotinoid insecticide thiamethoxam. *Bull Environ Contam Toxicol*. 80:431–437. <https://doi.org/10.1007/s00128-008-9420-y>.
- Hilton MJ, Emburey SN, Edwards PA, Dougan C, Ricketts DC. 2018. The route and rate of thiamethoxam soil degradation in laboratory and outdoor incubated tests, and field studies following seed treatments or spray application. *Pest Manag Sci*. 75(1):63–78. <https://doi.org/10.1002/ps.5168>.
- Huynh K, Leonard E, Chong JH, Palmer C, Tharayil N. 2021. Persistence and metabolism of the diamide insecticide cyantraniliprole in tomato plants. *Sci Rep*. 11(1):21570. <https://doi.org/10.1038/s41598-021-00970-8>.
- Johnson RM, Ellis MD, Mullin CA, Frazier M. 2010. Pesticides and honeybee toxicity—USA. *Apidologie*. 41:312–331. <https://doi.org/10.1051/apido/2010018>.
- Krischik V, Rogers M, Gupta G, Varshney A. 2015. Soil-applied imidacloprid translocates to ornamental flowers and reduces survival of adult *Coleomegilla maculata*, *Harmonia axyridis*, and *Hippodamia convergens* Lady Beetles, and Larval *Danaus plexippus* and *Vanessa cardui* butterflies. *PLoS One*. 10(3). <https://doi.org/10.1371/journal.pone.0119133>.
- Lentola A, David A, Abdul-Sada A, Tapparo A, Goulson D, Hill EM. 2017. Ornamental plants on sale to the public are a significant source of pesticide residues with implications for the health of pollinating insects. *Environ Pollut*. 228:297–304. <https://doi.org/10.1016/j.envpol.2017.03.084>.
- Li Z. 2022a. Spatiotemporal pattern models for bioaccumulation of pesticides in common herbaceous and woody plants. *J Environ Manage*. 276:111334. <https://doi.org/10.1016/j.envman.2020.111334>.
- Li Z. 2022b. Modeling pesticide residues in nectar and pollen in support of pesticide exposure assessment for honeybees: A generic modeling approach. *Ecotoxicol Environ Saf*. 236:113507. <https://doi.org/10.1016/j.ecoenv.2022.113507>.
- Loram A, Thompson K, Warren PH, Gaston KJ. 2008. Urban domestic gardens (XII): The richness and composition of the flora in five UK cities. *J Veg Sci*. 19(3):321–330. <https://doi.org/10.3170/2007.8-18373>.
- Long EY, Krupke CH. 2016. Non-cultivated plants present a season-long route of pesticide exposure for honeybees. *Nat Commun*. 7:11629. <https://doi.org/10.1038/ncomms11629>.
- Lowenstein DM, Matteson KC, Minor ES. 2019. Evaluating the dependence of urban pollinators on ornamental, non-native, and 'weedy' floral resources. *Urban Ecosyst*. 22:293–302. <https://doi.org/10.1007/s11252-018-0817-z>.
- Lu C, Hung YT, Cheng Q. 2020. A review of sublethal neonicotinoid insecticides exposure and effects on pollinators. *Curr Pollut Rep*. 6:137–151. <https://doi.org/10.1007/s40726-020-00142-8>.
- Mach BM, Bondarenko S, Potter DA. 2017. Uptake and dissipation of neonicotinoid residues in nectar and foliage of systemically treated woody landscape plants. *Environ Toxicol Chem*. 9999:1–11. <https://doi.org/10.1002/etc.4021>.
- Mundy-Heisz KA, Prosser RS, Raine NE. 2022. Acute oral toxicity and risks of four classes of systemic insecticide to the Common Eastern Bumblebee (*Bombus impatiens*). *Chemosphere*. 295. <https://doi.org/10.1016/j.chemosphere.2022.133771>.
- Pilling E, Campbell P, Coulson M, Ruddle N, Tornier I. 2013. A four-year field program investigating long-term effects of repeated exposure of honeybee colonies to flowering crops treated with thiamethoxam. *PLoS One*. 8(10). <https://doi.org/10.1371/journal.pone.0077193>.
- Pervez M, Manzoor F. 2020. Analysis of pesticide residues in pollen and nectar samples from various agricultural areas of Pakistan through high performance liquid chromatography. *SJA*. 36(1):1–9. <https://doi.org/10.17582/journal.sja/2020/36.1.1.9>.
- Rhodes LA, McCarl BA. 2020. An analysis of climate impacts on herbicide, insecticide, and fungicide expenditures. *Agronomy*. 10(5):745. <https://doi.org/10.3390/agronomy10050745>.
- Rollings R, Goulson G. 2019. Quantifying the attractiveness of garden flowers for pollinators.

- J Insect Conserv. 23:803–817. <https://doi.org/10.1007/s10841-019-00177-3>.
- Sanchez-Bayo F, Goka K. Pesticide residues and bees—a risk assessment. 2014. PLoS One. 9(4): E94482. <https://doi.org/10.1371/journal.pone.0094482>.
- Stanley DA, Garratt MPD, Wickens JB, Wickens VJ, Potts SG, Raine NE. 2015a. Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees. Nature. 528:548–550. <https://doi.org/10.1038/nature16167>.
- Stanley DA, Smith KE, Raine NE. 2015b. Bumblebee learning and memory is impaired by chronic exposure to a neonicotinoid pesticide. Sci Rep. 5:16508. <https://doi.org/10.1038/srep16508>.
- Stanley DA, Raine NE. 2017. Bumblebee colony development following chronic exposure to field-realistic levels of the neonicotinoid pesticide thiamethoxam under laboratory conditions. Sci Rep. 7:8005. <https://doi.org/10.1038/s41598-017-08752-x>.
- Stewart SD, Lorenz GM, Catchot AL, Gore J, Cook D, Skinner J, Mueller TC, Johnson DR, Zawislak J, Barber J. 2014. Potential exposure of pollinators to neonicotinoid insecticides from the use of insecticide seed treatments in the midsouthern United States. Environ Sci Technol. 48:9762–9769. <https://doi.org/10.1021/es501657w>.
- Stoner KA, Cowles RS, Nurse A, Eitzer BD. 2019. Tracking pesticide residues to a plant genus using palynology in pollen trapped from honeybees (Hymenoptera: Apidae) at ornamental plant nurseries. Environ Entomol. 48(2): 351–362. <https://doi.org/10.1093/ee/nvz007>.
- Suchail S, Guez D, Belzunces LP. 2000. Characteristics of imidacloprid toxicity in two *Apis mellifera* subspecies. Environ Toxicol Chem. 19: 1901–1905. <https://doi.org/10.1002/etc.5620190726>.
- Tong Z, Duan J, Wu Y, Liu Q, He Q, Shi Y. 2018. A survey of multiple pesticide residues in pollen and bee bread collected in China. Sci Total Environ. 640–641:1578–1586. <https://doi.org/10.1016/j.scitotenv.2018.04.424>.
- Torres JB, de F. Bueno A. 2018. Conservation biological control using selective insecticides – A valuable tool for IPM. Biol Control. 126:53–64. <https://doi.org/10.1016/j.biocontrol.2018.07.012>.
- Toumi K, Vleminckx C, van Loco J, Schiffrs B. 2016. Pesticide residues on three cut flower species and potential exposure of florists in Belgium. Int J Environ Res Public Health. 13(943). <https://doi.org/10.3390/ijerph13100943>.
- Tsvetkov N, Samson-Robert O, Sood K, Patel HS, Malena DA, Gajiwala PH, Maciukiewicz P, Fournier V, Zayed A. 2017. Chronic exposure to neonicotinoids reduces honeybee health near corn crops. Science. 356:1395–1397. <https://doi.org/10.1126/science.aam7470>.
- Vargas P, Liberal I, Ormosa C, Gómez JM. 2017. Flower specialisation: The occluded corolla of snapdragons (*Antirrhinum*) exhibits two pollinator niches of large long-tongued bees. Plant Biol. 19(5):787–797.
- Woodcock BA, Bullock JM, Shore RF, Heard MS, Pereira MG, Redhead J, Ridding L, Dean H, Sleep D, Henrys P, Peyton J, Hulmes S, Hulmes L, S'arospataki M, Saure C, Edwards M, Genersch E, Knabe S, Pywell RF. 2017. Country-specific effects of neonicotinoid pesticides on honeybees and wild bees. Science. 356:1393–1395. <https://doi.org/10.1126/science.aal1190>.
- Zhang X, Wang X, Liu Y, Fang K, Liu T. 2020. Residue and toxicity of cyantraniliprole and its main metabolite J9Z38 in soil-earthworm microcosms. Chemosphere. 249:126479. <https://doi.org/10.1016/j.chemosphere.2020.126479>.