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Special Research Report # 603: Impacts and Residual Longevity of Systemic Insecticides on Pollinators in Ornamental Plant Production System

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Insecticides in the chemical classes neonicotinoids, sulfoxaflor, flupyradifurone, diamides and tetramic acid derivatives may pose risks to pollinators that feed on nectar and pollen of treated plants. Floricultural crop growers need to understand that although these pollinators do not usually visit flowering crops grown in greenhouses, they can be exposed to insecticide residues when the plants are sold and transplanted in the landscape. One way to limit exposure to residues is to practice careful application timing and methods that could result in low residue concentration in pollen and nectar. Based on the results of this study, applications of cyantraniliprole, dinotefuran, imidacloprid, spirotetramat and sulfoxaflor could result in residue concentrations that are below the thresholds (25 ppb for nectar and 100 ppb for pollen) if the applications are conducted as foliar spray two or more weeks before sale or medium drench levels four or more weeks before sale. These results can be developed into guidelines for growers who wish to use these insecticides and protect pollinators. READ MORE…

BACKGROUND

Many growers have reduced or eliminated the use of neonicotinoids, a group of widely used systemic insecticides that have been perceived as detrimental to pollinators. Three systemic insecticides of different chemical classes are potential replacements for neonicotinoids: spirotetramat (Kontos), cyantraniliprole (Mainspring) and sulfoxaflor (XXpire, a product that also includes spinetoram). Limiting uses of neonicotinoids and the replacement of neonicotinoids with other systemic insecticides have significant economic and pest management implications to ornamental plant producers. Therefore, growers have ranked the examination of the potential impacts of these replacements, when applied under field-realistic use patterns and rates, on the survival and health of pollinators as a research priority.

Several recently published experiments, e.g. Cowles & Eitzer (2017; Journal of Environmental Horticulture 35: 24-34) and Mach et al. (2017; Environmental Toxicology & Chemistry 9999: 1-11), examined the behavior of neonicotinoids in ornamental crops. These studies, however, did not examine the impacts of neonicotinoids on pollinators, nor did they include spirotetramat, cyantraniliprole and sulfoxaflor in their studies.

The objectives of this project are 1) to document the translocation of imidacloprid, dinotefuran, spirotetramat, cyantraniliprole and sulfoxaflor to various plant tissues; 2) to quantify insecticide residues in the pollen, nectar and leaves of treated ornamental plants; and 3) to characterize mortality, behavior and colony health of honey bees after foraging on treated ornamental plants.

MATERIALS AND METHODS

Four ornamental plant species served as model plants in experiments conducted over two years at Clemson University, Pee Dee Research and Education Center, Florence, SC. In each experiment, plants were treated once in greenhouse with water (untreated check), and one of the following insecticides: Mainspring (cyantraniliprole) at 2 (foliar spray) and 12 fl oz/100 gal (medium drench), Safari 20SG (dinotefuran) at 4 (foliar) and 12 oz/100 gal (drench), Marathon II (imidacloprid) at 1.7 fl oz/100 gal (foliar) and 1.7 fl oz/3000 pots (drench), Kontos (spirotetramat) at 1.7 fl oz/100 gal (foliar) and 1.7 fl oz/3000 pots (drench), and XXpire (sulfoxaflor + spinetoram) at 2 fl oz/100 gal for both foliar and drench (drench is an off-label application). Foliar sprays were applied two weeks, and drenches were applied four weeks, before transplant into field plots.

Experiment 1: Residue concentrations in mountain mint (*Pycnanthemum muticum*)

Plugs were purchased from a nursery that did not use systemic insecticides. Plugs were treated, and transplanted, at periods described above. Leaf samples were collected from the plants in August and November 2015 (4 and 7 months after treatment, respectively) and August 2016 (1.1 year after treatment). Nectar samples were collected by centrifuging in August 2015 and 2016 (Figure 1).

Experiment 2: Residue concentrations in coneflower (*Rudbeckia lacinata*)

Plugs were purchased, grown, treated and transplanted as described for the mountain mint. Leaf samples were collected in May and August 2016 (1 month and 4 months after treatment). Pollen samples were collected by sieving in August 2016 (4 months after treatment). Insufficient number of coneflowers was available for collection of pollen and leaf samples in 2017.

Experiment 3: Residue concentrations in Salvia splendens

Plugs were donated by a collaborating greenhouse,and grown in 4-inch pots in the greenhouse in 2016 until saleable size. Plants were treated only with foliar sprays of systemic insecticides, and transplanted into the field plots two weeks after treatment. Nectar samples were collected by micropipeting 2, 5, 8 and 10 weeks after treatment.

Experiment 4: Residue concentrations in Portulaca x *hybrida*

Plugs were received, grown and treated as described for *Salvia*. Pollen samples were collected by sieving 2, 5 and 8 weeks after treatment in 2016.

Analysis of insecticide residue concentrations in leaf, nectar and pollen samples

Pollen (~100 mg per sample) and nectar (~500 µl per sample) were ground or prepared using liquid nitrogen and other solvents, and residue in the tissues were extracted with various solvents. Supernatants were vacuumed to dry, then reconstituted with acetonitrile. The extracted residues were analyzed with liquid chromatography tandem mass spectrometry (HPLC MS/MS). The analytical methodology and procedure are similar to those employed by Cowles and Eitzer (2017) and Mach et al. (2017), and are considered the most appropriate for detecting and quantifying small amount of insecticide residue in nectar and pollen.

Residue below the limit of detection (LOD) was considered to have a zero residue in this study, although it is possible that some very low levels of insecticide residues were present in nectar and pollen. LOD is the lowest residue concentration that is detectable by HPLC MS/MS.

We established toxicity thresholds of 25 ppb for nectar and 100 ppb for pollen for all plant species and insecticides. The 25 ppb threshold for nectar is the no observable effect level (NOEL) for colony

effects resulted from chronic exposure to nectar contaminated by imidacloprid (US EPA 2014, 2016). We follow the 100 ppb threshold for pollen proposed by Cowles and Eitzer (2017), which taken into consideration the NOEL for imidacloprid and the fact that worker bees consume more nectar than pollen.

Honey bee colony health and foraging behavior

Screen cages were erected on each field plot during peak bloom of mountain mints and *Rudbeckia* (Figure 2), and each received one honey bee nucleus (Figure 3). The nucleus was caged for 10 days, during which the numbers of bees foraging for 15 seconds in a 1-m^2 area (Figure 4), the numbers of dead bees discarded by the colony, and the numbers of surviving broods (eggs, larvae and pupae) in 100 marked cells (Figure 5) were recorded daily. About 470 ml of 25% sugar solution (w/w) was provided to each nucleus every other day to supplement resources provided by the flowers. The nuclei were moved to an organic farm and left opened for the 11th to 28th day (Figure 6), during which the numbers of surviving broods were recorded.

RESULTS

Residue concentrations in leaf, pollen and nectar samples

Mountain mint: Drench applications resulted in 59 ppb of imidacloprid residue and 52 ppb of spirotetramat, whereas spray application results in 28 ppb imidacloprid residue in nectar of mountain mint four months after treatment. These residue concentrations are above the toxicity threshold of 25 ppb. Residues of other insecticides were below the threshold at four months after treatment. Residues of all insecticides were not detected in the nectar one year after treatment.

Rudbeckia: None of the treatments resulted in residue concentrations exceeding the established threshold for pollen (100 ppb) at 4 months after treatment.

Salvia: Residue concentration exceeding the threshold for nectar (25 ppb) was detected 2 weeks after treatment only in plants treated with foliar spray of dinotefuran. Residue concentrations were below the threshold for all treatments at 5, 8 and 10 weeks after treatment.

Portulaca: None of the treatments resulted in residue concentrations exceedin the established threshold for pollen (100 ppb) at 2, 5 and 8 weeks after treatment.

Figure 1. Nectar extracted from mountain mint through centrifugation.

Figure 2. Mountain mints transplanted to the field plots were covered with screen cages.

Figure 3. Each field cage contained a honey bee nucleus.

Figure 4. Numbers of honey bees foraging in a 1-m² area were observed daily.

Figure 5. Survival of honey bee broods were determined by recording the numbers of live eggs, larvae and pupae in a 100-cell area over a 28-day period.

Figure 6. After the caging period, honey bees nuclei were moved to an organic farm and the bees were free to forage for 18 days. Brood survival was observed.

Honey bee colony health and foraging behavior

Honey bees caged in this portion of the study were exposed to nectar of mountain mint and pollen of *Rudbeckia* at four months after insecticide treatment.

Mountain mint: After being exposed for 10 days, the hive weights (including hive body and other hardwares) were on average 21 lbs 7 oz, and were not significantly different before and after the caging period. Colonies exposed to treated plants lost 50-143 bees per day. The numbers of foraging bees were not different among the treated and untreated plants. Honey bee broods exposed to treated and untreated plants survived poorly during the 28-day experimental period. On average, 5.3% of the marked broods were alive by the end of the 10-day caged period, and only 7.7% survived by the 18-day open-field period.

Rudbeckia: Nuclei weighted on average 20 lbs 6 oz, and colonies lost on average 58 bees/day. The hive weights, numbers of bees lost, and the number of foraging bees were not significantly different among the nuclei exposed to treated and untreated plants. Brood survival was 95% and 68%, respectively, at the beginning and the end of the caged period, and 57% at the end of the 18-day open-field period.

CONCLUSIONS

Foliar sprays and medium drench of the five systemic insecticides tested in this study did not result in residue concentrations exceeding the toxicity thresholds for pollen (100 ppb) and nectar (25 ppb) in *Rudbeckia*, *Salvia* and *Portulaca*. However, medium drench of imidacloprid and spirotetramat, and foliar spray of imidacloprid, resulted in residue concentration exceeding the threshold in the

nectar of mountain mint. Results of this study suggest that the degradation dynamic of systemic insecticides is likely different among plant species, even among herbaceous annual and perennial plants. Regardless of plant species, residues were not detected one year after treatment.

Results also suggest that application timing of at least two weeks before sale for foliar spray and at least four weeks before sale for medium drench will result in below-threshold residue in *Rudbeckia, Salvia* and *Portulaca*. For mountain mint, and similar nectar-producing herbaceous perennials, the spray and drench applications should be conducted more than two and four weeks before sale, respectively. We did not investigate the timing necessary to produce below-threshold residue concentrations in mountain mint.

We did not observe negative impacts of exposure to treated mountain mints and *Rudbeckia* on the average hive weight, adult bee survival, and adult bee foraging behavior. Results suggest that insecticide treatments in this study did not have significant impact on the survival and behavior of honey bees when compared to the untreated plants. Brood survival of the honey bee nuclei in all plants and insecticide treatments were very poor in this study, hindering our ability to make a conclusion on the impact of systemic insecticide residue on colony survival and health. The poor brood survival was likely a result of not providing sufficient amount of resources (nectar and pollen) in a small field plots (18.4 m^2 or about 80 plants in each plot) during the 10-day caged period. Once scarce resources negatively impacted brood survival during the first 10 days, the broods did not survive well during the next 18-day open-field phase.

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