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Research

Tracking Pesticide Residues to a Plant Genus Using Palynology in Pollen Trapped from Honey Bees (Hymenoptera: Apidae) at Ornamental Plant Nurseries

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Abstract

Worldwide studies have used the technique of pollen trapping, collecting pollen loads from returning honey bee (*Apis mellifera* L.) (Hymenoptera: Apidae) foragers, to evaluate the exposure of honey bees to pesticides through pollen and as a biomonitoring tool. Typically, these surveys have found frequent contamination of pollen with multiple pesticides, with most of the estimated risk of acute oral toxicity to honey bees coming from insecticides. In our survey of pesticides in trapped pollen from three commercial ornamental plant nurseries in Connecticut, we found most samples within the range of acute toxicity in a previous state pollen survey, but a few samples at one nursery with unusually high acute oral toxicity. Using visual sorting by color of the pollen pellets collected in two samples from this nursery, followed by pesticide analysis of the sorted pollen and palynology to identify the plant sources of the pollen with the greatest acute toxicity of pesticide residues, we were able to associate pollen from the plant genus *Spiraea* L. (Rosales: Rosaceae) with extraordinarily high concentrations of thiamethoxam and clothianidin, and also with high concentrations of acephate and its metabolite methamidophos. This study is the first to trace highly toxic pollen collected by honey bees to a single plant genus. This method of tracking high toxicity pollen samples back to potential source plants could identify additional high-risk combinations of pesticide application methods and timing, movement into pollen, and attractiveness to bees that would be difficult to identify through modeling each of the contributing factors.

Key words: pollen trapping, *Spiraea*, neonicotinoid, acephate, pollen hazard quotient

Over the history of pesticide use in the United States and around the world, the potential for pesticides, particularly insecticides, to harm honey bees has been repeatedly demonstrated (Johansen 1977, Johansen et al. 1983, Anderson and Wojtas 1986, Mineau et al. 2008). Honey bees can be exposed to pesticides through many different routes, including application of miticides inside their hives, contact with foliar applications in the air or on surfaces, contamination of water sources, contamination of nesting materials, and contamination of nectar and pollen (Sanchez-Bayo and Goka 2014).

The vastly increased use in recent years of nitroguanidine neonicotinoids, insecticides highly toxic to bees, including imidacloprid, thiamethoxam, clothianidin, and dinotefuran (Douglas and Tooker 2015), and the demonstration that these systemic insecticides can travel into the pollen and nectar of plants (Stoner and Eitzer 2012, Godfray et al. 2014, Godfray et al. 2015) have increased the interest worldwide in monitoring pollen as a potential source of exposure to honey bees and other bees.

Two methods have been used to collect substantial amounts of pollen for analysis of pesticide residue: trapping pollen from foragers as they return with pollen loads to the hive (Chauzat et al. 2006, Chauzat et al. 2011, Stoner and Eitzer 2013, Lu et al. 2015, Niell et al. 2015, David et al. 2016, de Oliveira et al. 2016, Long and Krupke 2016, Alburaki et al. 2017, Colwell et al. 2017, Nai et al. 2017, Smart et al. 2017, Böhme et al. 2018, Drummond et al. 2018, Prado et al. 2018, Tosi et al. 2018) and collecting stored pollen or bee bread inside the hive (Škerl et al. 2009, Bernal et al. 2010, Lawrence et al. 2016, Traynor et al. 2016, McArt et al. 2017).

In general, these surveys have found complex mixtures of pesticides in honey bee pollen, including herbicides, fungicides, and insecticides. One way to evaluate the acute toxicity of each pesticide is to scale the pesticide residue concentration according to the acute oral toxicity of the pesticide to adult worker honey bees (oral LD_{50}), creating a Pollen Hazard Quotient (Stoner and Eitzer 2013, Traynor et al. 2016). If the pesticides are assumed to be additive in effect, the Pollen Hazard Quotients for each pesticide in a sample can be added

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to create an overall Pollen Hazard Quotient for the sample (Traynor et al. 2016). This is a simplifying assumption and likely to be conservative, given that certain fungicides and insecticides are known to interact synergistically (Pilling and Jepson 1993, Iwasa et al. 2004, Thompson et al. 2014) and some may have a time-cumulative effect over the adult life of the bee (Sanchez-Bayo and Goka 2014, Holder et al. 2018). However, it does allow comparison of the potential for acute oral toxicity over the wide range of pesticides found in pollen in the absence of detailed quantitative information on all possible interactions or cumulative effects.

The Pollen Hazard Quotient puts pesticide concentration into the context of acute oral toxicity to honey bees, and does not address the wide range of sublethal effects of many insecticides (Desneux et al. 2007, Alkassab and Kirchner 2017), and fungicides (Prado et al. 2018) on honey bees, even at relatively low levels frequently found in the field. Sublethal effects of neonicotinoids at low mean concentrations frequently found in trapped pollen in the field have been demonstrated at the levels of genes, immune function, neurology, and behavior of individual worker bees (Alkassab and Kirchner 2017). However, effects on honey bee colonies as a superorganism (Straub et al. 2015) have been subtle and inconsistent in colonies fed neonicotinoids in pollen over a period of years (Sandrock et al. 2014a) even at levels up to 100 ppb (parts per billion, or µg/kg), a concentration rarely found in mixed pollen in the field (Dively et al. 2015).

Pesticides in honey bee pollen have been surveyed in many environments, comparing agricultural regions within a country (Chauzat et al. 2006, Chauzat et al. 2011, Tosi et al. 2018); or urban, rural, and agricultural areas within a state (Stoner and Eitzer 2013, Lawrence et al. 2016, Alburaki et al. 2017, Drummond et al. 2018); or in crop or orchard sites with different pesticide practices (Long and Krupke 2016, McArt et al. 2017). This study differs from previous pollen surveys in our focus on large commercial nurseries producing ornamental plants, and our use of color sorting and palynology to identify the plant source of pollen with high pesticide residues.

Public concern about pesticide residues in pollen and nectar of ornamental plants, particularly with respect to nitroguanidine neonicotinoids, has come primarily from the work of environmental organizations. Publications of the Xerces Society for Invertebrate Conservation highlighted concerns about the lack of data on residues of nitroguanidine neonicotinoids in pollen and nectar of ornamental plants, despite the higher rates and different application methods used in treating ornamental plants in comparison to food crops, and the evidence that neonicotinoids can persist for years in woody plants (Hopwood et al. 2012, Hopwood et al. 2016). A survey by Friends of the Earth of plants purchased at retail garden centers in the United States and Canada found widespread residues of neonicotinoids in leaves, stems, and flowers of ornamental plants, often at alarming levels, such as when thiamethoxam (754 ppb), clothianidin (76.9 ppb), and imidacloprid (78.0 ppb) were all three found in the flowers of African daisy at one site (Brown et al. 2014). However, this survey did not measure pesticides in nectar or pollen, presumably because of the difficulty in collecting quantities suitable for analysis, so it is uncertain how these concentrations in flowers would relate to those directly affecting bees. A follow-up survey of retail garden centers in the United States by Friends of the Earth found reduced incidence of neonicotinoid residues in plants purchased at garden centers, but still some specific instances of high concentrations of nitroguanidine neonicotinoids in flowers: up to 889 ppb of imidacloprid, 64.2 ppb dinotefuran, and 82.5 ppb thiamethoxam, not all in the same sample (Kegley et al. 2016). As before, nectar and pollen were not analyzed. The reductions in neonicotinoid incidence were attributed to consumer pressure on retailers (Kegley et al. 2016).

Lentola et al. (2017) also studied pesticide residues in plants at retail garden centers, in this case in the United Kingdom. In addition to measuring residues in leaves, they also measured residues in pollen and nectar. The few nectar samples they were able to collect had generally low concentrations of pesticides, with most below their limit of quantification, but pollen samples included higher concentrations of thiamethoxam and clothanidin (mean for both = 11.0 ppb) than the range (2–6 ppb) considered to be field-realistic based on research on seed-treated crop plants (Godfray et al. 2015).

These publications and the attendant publicity have raised concern among the public, the nursery growers, and the Connecticut Department of Energy and Environmental Protection, the funding agency for this study. The nursery and greenhouse industries are by far the largest agricultural industries by value in Connecticut, with combined annual sales of $253 million, representing 46% of the market value of all Connecticut agricultural products in the National Agricultural Statistics Survey of 2012 (US Dept. Department of Agriculture 2017). There are, of course, multiple sites in the production and distribution system where ornamental plants could be exposed to pesticides, including at retail garden centers as well as at nursery production operations, but this study focuses specifically on production nurseries.

The objective of this study was to measure pesticide residues in trapped pollen from commercial nurseries in Connecticut specializing in ornamental plant production in order to determine whether levels of systemic pesticides in nursery pollen may pose a risk to honey bees and other pollinators. Another objective, arising from our finding of unusually high acute toxicity levels during a short time at one of the commercial nurseries, was to determine from what plant species this pollen originated.

Materials and Methods

Study Sites

The study sites were three wholesale plant nurseries in Connecticut, separated from each other by a minimum of 42 km. Nursery C was the smallest of the nurseries, with an area of 48 ha, located in south-central Connecticut, 2.4 km from Long Island Sound, and surrounded by forest interspersed with suburban development. Nursery M encompassed an area of 183 ha in north-central Connecticut, surrounded by agricultural fields, suburban development and forest. Nursery P has an area of 168 ha, in a more rural area of eastern Connecticut with primarily agricultural fields mixed with forest.

Pollen Collection

Nine honey bee hives (*Apis mellifera* L., Carniolan race) (Hymenoptera: Apidae), were started from packages (containing 1.4 kg of worker bees) with mated queens on new 10-frame Langstroth equipment, allowed to establish and feed at the CT Agricultural Experiment Station Lockwood Farm (Hamden, CT, 06518), using a bucket feeder with sugar water (1:1) supplemented with frames of honey and pollen collected the previous year at Lockwood Farm. Three hives were moved to each of the three commercial ornamental plant nurseries on 8 May 2015. Each colony was set up at the nurseries with two deep hive boxes with plastic frames for each colony with screened bottom board, inner cover, and telescoping lid, elevated on pallets, and placed in a location chosen to be near a water source and within the nursery area but not likely to be directly sprayed. All hives had Sundance bottom-mounted pollen

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traps (Ross Rounds, Inc., Canandaigua, NY). Hives were inspected weekly at the time of pollen collection to determine whether they were queenright, and to make sure that the bees were traveling through the pollen trap rather than through alternate entrances. No treatments for mites or disease were applied during pollen collection. Replacement queens from the same source were added as needed, and additional supers were also added as needed.

Pollen traps collect pollen by forcing returning foragers to travel through a screen that allows the worker bee to go through, but detaches the two pollen loads she carries in her pollen baskets. The detached pollen loads (referred to below as pollen pellets) fall vertically through another screen that keeps out larger debris, and are collected above a finer screen that allows fine debris to drop out and permits ventilation, ideally resulting in a sample of pollen pellets, each representing a pollen load from one forager trip, of fairly uniform size. Pollen samples were collected from 8 May until 23 September 2015.

In order to allow the honey bees to collect some pollen for their own use, each hive was set to trap pollen for 2 wk and then set on bypass to allow the bees to store pollen in the hive in the third week. The hives that were collecting or on bypass were rotated each week so that at any given time two of the three hives were collecting pollen. Pollen was collected weekly from the pollen traps, and the pollen from each of the two hives was collected, stored, and analyzed separately. Pollen was collected into 50 ml centrifuge vials for up to three vials per sample, and the excess beyond that was stored in plastic bags. Pollen was frozen in standard freezers (approximately –20°C) immediately upon return to the laboratory and stored in the same freezers until use.

Pesticide Analysis

The standard procedure extracted samples using a modified version of the QuEChERS (for Quick, Easy, Cheap, Effective, Rugged, and Safe) protocol (Anastassiades and Lehotay 2003). In brief, 5 g pollen was spiked with 100 ng of isotopically labeled (d-4) imidacloprid (Cambridge Isotope Laboratories, Tewksbury, MA) as an internal standard. Water was added to achieve a total mass of 15 g. After mixing, 15 ml of acetonitrile (Pesticide Grade, Thermo Fisher Scientific, Waltham, MA), 6 g magnesium sulfate and 1.5 g sodium acetate were added. After shaking and centrifuging, 10 ml of the supernatant was combined with 1.5 g magnesium sulfate, 0.5 g primary and secondary amine exchange material (PSA Bonded Silica, Sigma Aldrich, St. Louis, MO), 0.5 g 18-carbon length silica-bound sorbent (Discovery DSC-18, Sigma Aldrich), and 2 ml toluene. The samples were shaken and centrifuged and 6 ml of the supernatant was concentrated to 1 ml for instrumental analysis. Two different LC–MS systems were utilized:

System 1: A Dionex 3000 LC interfaced to a Thermo Velos Pro Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA) using an Agilent SB-C18-RRHD-2.1 mm × 150 mm column packed with 1.8 µm particles (Santa Clara, CA), using water with 0.1% acetic acid as mobile phase A and acetonitrile with 0.1% formic acid as phase B. The gradient series was 95% A until 1 min, 60% A at 6 min, 5% A at 20 min, hold for 5 min, and then re-equilibrated at 95% A for 4 min. This instrument was operated in both positive and negative electrospray ionization modes. As this instrument is a unit resolution mass spectrometer, a unique scan function was used for each monitored pesticide to enhance specificity and to generate tandem mass spectroscopy data for that pesticide, which was then used for quantitation (except for a couple of pesticides for which the mass of molecular fragments could not be determined).

System 2: An Agilent 1200 LC interfaced to a Thermo Exactive Mass spectrometer using a Hypersil Gold-aQ C-18-2.1 mm × 100 mm column packed with 1.9 µ particles (Thermo Fisher Scientific), using water with 0.1% acetic acid as mobile phase A and acetonitrile with 0.1% formic acid as phase B. The LC used a gradient series of 99% A until 1 min, 5% A at 20 min, hold for 3 min, and then re-equilibrated at 95% A for 4 min. Again, the instrument was operated in both positive and negative electrospray modes. This instrument is a high resolution mass spectrometer so only three scan functions were used: a full scan at resolution of 50,000 and two all-ion fragmentation scans at 25,000 resolution. Pesticides were quantitated using a 5 ppm window around the primary ion in the full scan.

The Velos Pro was the primary quantitation instrument while the Exactive was used for confirmation of pesticide residues or residues not in the Velos method.

Not all pesticides would be detected with the analytical methods used. In particular, the pyrethroids bifenthrin, cyfluthrin, and permethrin and the macrocyclic lactone abamectin, which were applied at the nurseries according to their pesticide records, would not be detected. (Bifenthrin was used by the nurseries mainly in granular form applied to potting soil for grub control.) In addition, the fungicide chlorothalonil would not be detected unless present in large amounts, although the metabolite 4-hydroxychlorothalonil can be and was detected.

Calculation of Pollen Hazard Quotient and Estimated % LD_{50}

For each pesticide detected in a sample, Pollen hazard quotients and estimated LD_{50} were calculated using the methods of Stoner and Eitzer (2013):

$$\text{Pollen hazard quotient} = \frac{\text{concentration in } \mu\text{g/kg}}{\text{oral } LD_{50}}$$

$$\text{Estimated } LD_{50} = \frac{\text{conc. in } \mu\text{g/kg} \times (\text{nurse bee consumption of pollen per day})}{\text{oral } LD_{50}}$$

Oral LD_{50} values came from Sanchez-Bayo and Goka (2014). For pesticides where only a lower limit on the LD_{50} was determined, that lower limit was used in calculations. (Generally, this meant that the LD_{50} was > 100 µg/bee.) Assuming that the acute toxicity effects of the pesticides were additive, rather than synergistic, we summed the Pollen Hazard Quotients for each pesticide in the sample to get an overall Pollen Hazard Quotient for the sample. Using the average daily pollen consumption of a nurse bee of 9.5 mg/bee/day (Crailsheim et al. 1992, Rortais et al. 2005), a Pollen Hazard Quotient of 1,000 corresponds to consuming 1% of the oral LD_{50} per day (Stoner and Eitzer 2013, Traynor et al. 2016, Tosi et al. 2018).

Pollen Sorting and Analysis of Color Categories

A subsample of pollen pellets from the selected trapped pollen samples was sorted into visual categories in a well-lit laboratory on an off-white laboratory bench. The sorted color categories were assigned names and numbers by visual comparison to the Pantone Fashion + Home Color Guide TPX (Design Info 2013). Ten pellets from each color category were set aside for pollen identification, and the remaining amount was re-analyzed for pesticide residues, using the same methods as earlier. Because some experimental methods to prepare samples for palynology were unsuccessful, in some cases

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only five pellets remained for traditional acetolysis and palynology, as described in what follows.

Palynology

For the selected samples that were sorted by color (Nursery C, hive C, 17 August and 24 August), either 5 or 10 pollen pellets (depending on availability) from each color-sorted sample were analyzed. Each pollen pellet was prepared separately with acetolysis at the Connecticut Agricultural Experiment Station Valley Laboratory in Windsor, CT and followed procedures outlined in Faegri et al. (1989). Pellets were disarticulated with 10% hydrochloric acid, and glacial acetic acid washes removed all water in the samples before and after acetolysis. A 9:1 mixture of acetic anhydride and sulfuric acid removed cellular contents and the cellulose wall. The pollen sample was then dehydrated with 95% ethanol and suspended in silicone oil for storage. Samples for pollen analysis were mounted on glass slides under 23 mm^2 coverslips and sent to the Climate Change Institute, University of Maine, Orono, Maine, for pollen identification.

For each sample slide, the entire slide was scanned and all pollen types identified to plant family, genus, or species with the greatest specificity possible. Pollen cells were counted along three separate transects until all pollen species were recorded and a minimum of 300 pollen cells counted. Pollen was identified using standard keys (Faegri et al. 1989, Crompton and Wojtas 1993, McAndrews et al. 2005), pollen reference samples collected on-site at the nurseries, the extensive pollen reference collection at the Climate Change Institute, and PalDat - Palynology Database (https://www.paldat.org). In most cases, pollen was identified to genus, but some pollen types were identified only to family (e.g., Caryophyllaceae). Where possible, pollens were identified to species (e.g., *Trifolium pratense* and *T. repens*).

The composition by count of the pollen from each color-sorted sample was calculated using the percentage species composition in the counts for each pellet divided by the total number of pellets analyzed. To calculate the composition of the sample by weight, we assumed that the weight of a pollen grain is proportional to volume. We measured the polar and equatorial dimensions of a representative sample of pollen grains of a particular species, and then calculated the volume using standard formulae for a prolate or oblate sphere. Species percentages by count were then weighted according to volume.

Statistical Analysis

The relationships among pesticide concentrations (in ppb) and pollen species (as proportion of each species by weight) were analyzed by calculating the Pearson product–moment correlation coefficient (SystatSoftware 2018) for the color-sorted pollen from the highest toxicity pollen sample (Nursery C, hive C, 17 August 2015). Non-detections of pesticides and nondetections of *Spiraea* pollen were treated as zeros in the analysis.

Results

Pesticide Residues at All Three Nurseries

The summed Pollen Hazard Quotients, adding together the individual Pollen Hazard Quotients for each pesticide in a weekly pollen sample trapped from a single hive, were estimated to be below 5% of the honey bee LD_{50} for all the pollen samples at Nurseries M and P and most of the pollen samples (87%) from Nursery C (Table 1 and Fig. 1). As shown in Fig. 1, most of the pollen samples at Nurseries M and P were below 1% of the honey bee LD_{50}, as was also true of Nursery C (Supplementary Information [online only]).

The major pesticides adding to the Pollen Hazard Quotients were the nitroguanidine neonicotinoids (imidacloprid, thiamethoxam, and its metabolite clothianidin) followed by the organophosphate acephate and its metabolite methamidophos (Table 2).

The three nurseries differed in the pattern of neonicotinoids detected. Thiamethoxam and its metabolite clothianidin were the major neonicotinoids detected at Nursery C (Table 2). Imidacloprid was found in only one sample at Nursery C, at a concentration of 2.5 ppb (Table 2). At Nursery C, thiamethoxam and its metabolite clothianidin were found only in samples from the month of August. The high concentrations of these two neonicotinoids, and also of the organophosphate acephate and its metabolite methamidophos, were the major contributors to the high Pollen Hazard Quotients and high acute toxicity relative to the honey bee LD_{50} of these samples (discussed further below).

At Nursery P, thiamethoxam was present only rarely (6.5%) and at relatively low levels (mean and median 3.9 ppb), and likewise clothianidin was found in only a single sample at Nursery P at 4.4 ppb (Table 2). Neither thiamethoxam nor clothianidin were detected at all at Nursery M. At Nursery M, imidacloprid was found frequently (54.3% of samples) with mean and median concentrations of 3.9 and 3.8 ppb, respectively, and a maximum concentration of 9.9 ppb (Table 2). At Nursery P, imidacloprid was found in 32.3% of samples with a mean of 2.9 and median of 2.7 ppb.

The organophosphate acephate and its metabolite methamidophos were found in samples at all three nurseries, even though the limit of detection for each of these insecticides was relatively high (20 ppb for acephate and 5 ppb for methamidophos) compared with most other insecticides (Table 2). Although the oral LD_{50} for these organophosphates are 15–40× higher than those of the nitroguanidine neonicotinoids, the mean concentrations of acephate at Nurseries M and P were also 15–38× higher than those of the nitroguanidine neonicotinoids, the mean concentrations of methamidophos were 2.5–7.5× higher, and the maximum Pollen Hazard

Table 1. Estimated acute toxicity of trapped pollen collected at three commercial nurseries in Connecticut in 2015, based on summing the Pollen Hazard Quotients (PHQ) of each pesticide in the weekly pollen sample, and then estimating percentage of honey bee LD_{50} by defining 1,000 PHQ units as 1% of the honey bee LD_{50}

Site	No. of pollen samples	Mean pollen hazard quotient	Median pollen hazard quotient	No. of samples below 5% of honey bee LD_{50}	No. of samples between 5 and 10% of honey bee LD_{50}	No. of samples above 10% of honey bee LD_{50}
M	35	397	231	35	0	0
P	31	231	36	31	0	0
C	38	3,985	71	33	2	3

Pollen samples were collected from two hives each week, when adequate pollen for analysis was trapped by the bees.

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Table 2. Insecticides and fungicides in trapped pollen collected at three commercial nurseries in Connecticut in 2015

	LOD	Oral LD_{50} (µg/bee)	Nursery C				PHQ of max	Nursery M				PHQ of max	Nursery P				PHQ of max
			% detected	Mean (ppb)	Median (ppb)	Max ppb		% detected	Mean (ppb)	Median (ppb)	Max ppb		% detected	Mean (ppb)	Median (ppb)	Max ppb	
Insecticides/Acaricides																	
Acetamiprid	2	14	ND					ND					3.2%	1.6	1.6	1.6	0.11
Clothianidin	2	0.0035	23.7%	17.3	4.5	78	22,285	ND					3.2%	4.4	4.4	4.4	1,257
Imidacloprid	2	0.013	2.6%	2.5	2.5	2.5	192	54.3%	3.9	3.8	9.9	762	32.3%	2.9	2.7	5.1	392
Imidacloprid, 5- Hydroxy	2	0.013	ND					ND					3.2%	2.0	2.0	2.0	154
Thiamethoxam	2	0.005	23.7%	53.9	7.8	305	61,000	ND					6.5%	3.9	3.9	4.4	880
Acephate	20	0.23	28.9%	115	94	394	1,713	37.1%	59	29	242	1,052	6.5%	110	110	194	843
Carbaryl	2	0.15	ND					5.7%	82.8	82.8	164	1,093	ND				
Chlorpyrifos	2	0.24	7.9%	4.3	4.1	5.0	20.83	37.1%	7.0	4.9	22	91.67	6.5%	1.3	1.3	1.5	6.25
Coumaphos	1	4.6	ND					2.9%	3.1	3.1	3.1	0.67	ND				
Diazinon	0.5	0.21	7.9%	0.6	0.6	0.9	4.29	ND					16.1%	0.9	0.8	2.1	10
Dimethoate	1	0.17	ND					8.6%	2.5	2.7	3.6	21.18	ND				
Flubendiamide		>200	7.9%	3.2	1.1	7.4	0.04	ND					ND				
Methamidophos	5	0.2	42.1%	50	18	390	1,950	60.0%	29.5	17.0	155	775	38.7%	11	6	49	245
Methiocarb	1	0.47	ND					ND					3.2%	18.0	18.0	18.0	38
Phosmet	5	0.37	ND					2.9%	11.4	11.4	11.4	30.81	ND				
Spiromesifen	2	790	ND					5.7%	254.5	254.5	456	0.58	ND				
Spirotetramat	1	195	ND					2.9%	3.4	3.4	3.4	0.02	ND				

	LOD	Oral LD50 (µg/bee)	Nursery C				PHQ of max	Nursery M				PHQ of max	Nursery P				PHQ of max
			% detected	Mean (ppb)	Median (ppb)	Max ppb		% detected	Mean (ppb)	Median (ppb)	Max ppb		% detected	Mean (ppb)	Median (ppb)	Max ppb	
Fungicides																	
Azoxystrobin	1	>25	71.1%	13.3	0.6	294.0	11.76	51.4%	2.8	1.1	26.0	1.04	19.4%	0.8	0.4	2.7	0.11
Boscalid	1	166	65.8%	30.9	5.5	240.0	1.45	57.1%	96.9	3.7	1,693.0	10.20	87.1%	16.0	4.5	215.0	1.30
Carbendazim	2	>50	100.0%	42.8	11.0	225.0	4.50	11.4%	6.8	6.2	14.0	0.28	96.8%	104.8	8.6	1,383.0	27.66
Dimethomorph	0.5	32	ND					37.1%	190.6	2.2	1,823.0	56.97	9.7%	1.8	1.9	2.6	0.08
Fludioxonil	3	329	ND					20.0%	21.6	9.0	90.0	0.27	ND				
4-Hydroxychloroth-alonil		63	71.1%	59.8	14.0	407.0	6.46	34.3%	28.4	7.3	132.0	2.10	58.1%	137.5	4.2	1,046.0	16.60
Iprodione	2	25	ND					ND					29.0%	416.8	240.0	1,526.0	61.04
Mandiproamide	1	>200	ND					17.1%	68.4	4.5	229.0	1.15	ND				
Metalaxyl	0.5	269	81.6%	1.2	0.8	6.2	0.02	5.7%	0.6	0.6	0.8	0.00	25.8%	0.9	0.9	1.7	0.01
Metconazole		87	ND					ND					6.5%	2.2	2.2	2.9	0.03
Myclobutanil	3	34	ND					51.4%	38.8	7.3	213.0	6.26	12.9%	51.4	9.7	185.0	5.44
Propiconazole	1	77	7.9%	12.1	13.0	15.0	0.19	22.9%	12.7	3.9	73.0	0.95	19.4%	5.6	2.0	22.0	0.29
Pyraclostrobin	1	73	42.1%	12.2	1.7	94.0	1.29	22.9%	70.9	1.7	486.0	6.66	77.4%	3.1	1.1	39.0	0.53
Tebuconazole	10	83	ND					ND					12.9%	131.9	96.5	327.0	3.94
Thiophan-ate-methyl	2	>100	60.5%	21.3	9.3	107.0	1.07	5.7%	9.8	9.8	17.0	0.17	54.8%	320.2	7.6	3,762.0	37.62
Trifloxystrobin	1	>200	ND					2.9%	25.0	25.0	25.0	0.13	ND				

LOD, limit of detection; ND, not detected; PHQ, Pollen Hazard Quotient; ppb, parts per billion. Means and medians are for those samples where the pesticide was detected (zeros were not included).

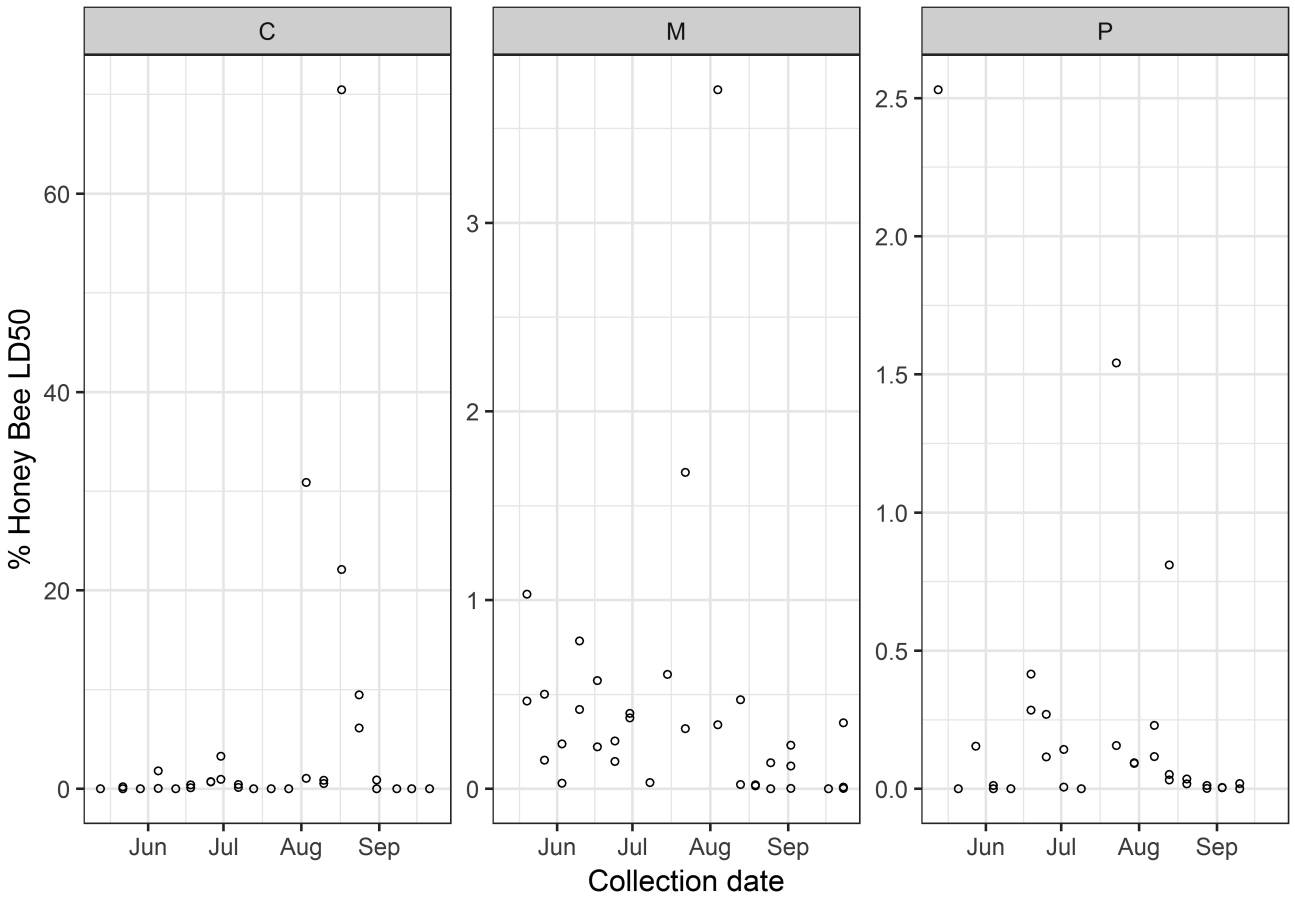


Fig. 1. Percentage of the honey bee LD_{50} from pesticides detected in pollen trapped from honey bee hives at three ornamental plant nurseries (labeled as Nurseries C, M, and P). Note the different scales on the Y-axis for each nursery.

Quotients were in a similar range for both groups of insecticides at these two nurseries (Table 2). The frequency of detection and mean and median concentrations of acephate and methamidophos at Nursery C were similar to those at Nursery M, although the maximum concentrations were higher.

The rest of the insecticides and acaricides had relatively little effect on Pollen Hazard Quotients, except for carbaryl, which was detected in only two samples at Nursery M, but one sample had a concentration of 164 ppb. With an oral LD_{50} of 0.15 µg/bee, the maximum Pollen Hazard Quotient for carbaryl was similar to that of acephate at the same nursery (Table 2).

In addition to insecticides and acaricides, 16 fungicides (Table 2) and 9 herbicides (Supplementary Information [online only]) were detected. As with the insecticides, the pattern of fungicide residues varied among the three nurseries. At Nursery P, 13 fungicides were detected, with thiophanate-methyl together with its metabolite carbendazim, iprodione, the chlorothalonil metabolite 4-hydroxy-cholorothalonil, and tebuconazole at relatively high concentrations. Nursery M also had 13 fungicides detected, with dimethomorph and boscalid at higher concentrations. At Nursery C, eight fungicides were found, all at relatively low concentrations.

High Pollen Hazard Quotient Samples at Nursery C

All of the samples with an estimated percentage of Honey Bee LD_{50} greater than 5% were trapped from Nursery C in August of 2015 (Table 3). These high Pollen Hazard Quotients were primarily due to thiamethoxam and clothianidin. Acephate and methamidophos together accounted for 1% or less of Honey Bee LD_{50} for these samples, and the other pesticides accounted for 0.01% or less. There was tremendous variation in the concentrations of thiamethoxam and clothianidin, and thus Pollen Hazard Quotients and % Honey Bee LD_{50}, between hives in the same apiary during the same week (e.g., 3 August) and between samples trapped a week apart from the same hive (e.g., hive B, 10 August and 17 August).

When the sample with the greatest acute toxicity, from hive C at Nursery C on 17 August 2015, was sorted into color categories and the categories were re-analyzed for pesticide residues, thiamethoxam, clothianidin, acephate, and methamidophos were concentrated by 2.2–6.5× in two of the 11 color categories compared with the original bulk sample and by 1.1–2.5× in one other color category (Table 4). Among the 15 pollen species found in all the color-sorted samples, only *Spiraea* pollen was closely correlated with each of the four insecticides (Table 5 and Supplementary Information [online only]). The concentrations of thiamethoxam, clothianidin, acephate, and methamidophos in relation to the proportion of *Spiraea* pollen by weight are illustrated in Fig. 2. Additional support for the relationship between *Spiraea* and thiamethoxam and clothianidin in pollen at Nursery C came from the color-sorted pollen from the same hive trapped 1 wk later (Nursery C, hive C, 24 August 2015). Even though the original bulk sample had only 3.7% *Spiraea* pollen, sorting the pollen by color concentrated the *Spiraea* pollen to 94.1% in a single color category, and concentrated the thiamethoxam and clothianidin by 11 and 15-fold, respectively (Table 6). Another additional line of evidence for the association of *Spiraea* pollen with high toxicity pesticide residues at Nursery C in August 2015 comes from another project

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Table 3. Insecticide residues (concentrations given in ppb) in trapped pollen samples from Nursery C in August 2015 (ND = not detected, ppb = parts per billion)

Date	Hive	Thiamethoxam	Clothianidin	Acephate	Methamidophos	Pollen Hazard Quotient	Estimated % Honey Bee LD_{50}
3 Aug.	A	2.5	2	ND	ND	1,072	1.1
3 Aug.	C	43	78	ND	ND	30,888	30.9
10 Aug.	A	1.0	1.2	ND	ND	543	0.5
10 Aug.	B	1.4	2	ND	ND	852	0.9
17 Aug.	B	81	20	31	15	22,125	22.1
17 Aug.	C	305	31	94	40	70,472	70.5
24 Aug.	A	41	4.5	ND	ND	9,486	9.5
24 Aug.	C	7.8	16	ND	ND	6,132	6.1
31 Aug.	A	ND	ND	ND	ND	7.7	0.01
31 Aug.	B	2.7	1.2	ND	ND	889	0.89

using palynology and molecular barcoding for analysis of bulk pollen. In two samples, from Hive A (10 August 2015) and Hive B (31 August 2015), both with relatively low concentrations of thiamethoxam and clothianidin and no detection of acephate or methamidophos (Table 3), no *Spiraea* pollen was detected by either method (Sponsler, personal communication.)

The pesticide records provided to us by Nursery C confirmed foliar applications of acephate (1 lb/100 gallon = 1.2 g/liter) to the salable crop of *Spiraea* on 2 June 2015, and Flagship 25 WG (4 oz./100 gallon = 0.3 g/liter of formulated product) to part of the crop to be held for further growth on 29 July 2015, and to the rest of the 'grow-on' crop on 12 August 2015.

Sorting Pollen by Color for Palynology and Pesticide Analysis

Although sorting pollen pellets by color in these two bulk samples concentrated the pesticide residues in a smaller portion of the original sample, and concentrated to 94% *Spiraea* pollen in a single color category in the Hive C 24 August sample (Table 6), we have not found a general one-to-one relationship between pollen plant origin and pellet color. For the most part, this was not due to substantial mixing of pollen types within a pollen pellet. With the exception of a few pollen pellets mixing *Zea mays* with other species, each individual pollen pellet was composed of >92% of a single dominant pollen type (Table 7). The problem was rather that pollen pellets dominated by the pollen from the same plant species may look different enough to sort into three different color categories (Table 7), or conversely, pellets sorted into the same color category may include pellets with three different dominant pollen species (e.g., 'butterscotch,' 'freesia,' 'yolk yellow,' 'straw,' and 'mustard gold' from Hive C 17 August sample, Supplementary Information [online only]).

Discussion

For most of the trapped pollen samples from these ornamental plant nurseries, the concentrations of nitroguanidine neonicotinoids fell within the 'field-realistic' range of 2–6 ppb based on reviews of many studies of nectar and pollen of seed-treated field crops and previous surveys of trapped pollen (Blacquiere et al. 2012, Godfray et al. 2014, Godfray et al. 2015). The only exceptions were five of the samples from Nursery C in August, as discussed earlier, and two samples from Nursery M with concentrations of imidacloprid of 7.2 and 9.9 ppb (Supplementary Information [online only]).

In our previous survey of trapped pollen in several locations in Connecticut, there was a much lower incidence of detection of nitroguanidine neonicotinoids [12% of samples detected imidacloprid, 4% thiamethoxam, and 1% dinotefuran, and no clothianidin (Stoner and Eitzer (2013)]. However, in most samples in the previous survey, the concentrations of these pesticides were in a similar range with the current study, with only one unusally high sample (70 ppb imidacloprid). Acephate and methamidophos were much more rarely detected in the previous study (1.9 and 0.3% detections, respectively). Other insecticides used in orchards and field agriculture, such as phosmet and carbaryl were found more frequently (Stoner and Eitzer 2013). Certain fungicides were found more frequently in this nursery study compared with our previous survey, including carbendazim, thiophanate-methyl, azoxystrobin, boscalid, pyraclostobin, and metalaxyl.

A previous survey of pesticides from 32 sites in Maine, ranging from unmanaged to managed agricultural and urban landscapes (Drummond et al. 2018), did not detect any nitroguanidine neonicotinoids, and also did not detect acephate or methamidophos, but did find the insecticides phosmet and carbaryl at a few sites. Another survey in Massachusetts, collecting pollen from 62 hives in 10 counties across the state and analyzing only neonicotinoids (Lu et al. 2015), found neonicotinoids more frequently than this study (72% of Massachusetts samples had at least one neonicotinoid, compared with 38% here), likely due in part to having much lower detection limits (0.1 ppb, compared with 2 ppb here). These samples, taken 1 day per month over 5 mo at each site, found some high concentrations of neonicotinoids (e.g., 25.2 ppb of imidacloprid-equivalent as a monthly average for one county). This mean presumably included substantial variation among sites within the county because the standard deviation was 3.3× the mean (Lu et al. 2015).

Our survey data are not comparable to those of Friends of the Earth (Brown et al. 2014, Kegley et al. 2016) or Lentola et al. (2017), because our sample of trapped pollen did not come directly from nursery plants, but would be expected to contain a mix of species from the wide geographical range covered by honey bee foragers. We are currently analyzing the bulk trapped pollen from all three nurseries through palynology and molecular barcoding to determine the plant sources over the season, and preliminary data indicate that a substantial fraction of the pollen comes from plant genera and families not grown by the nurseries (Sponsler, personal communication).

Others have used palynology to investigate whether the pesticide concentrations in honey bee pollen adjacent to a crop is related to the proportion of crop pollen or to noncrop plants in the area. Long and Krupke (2016) found very little honey bee collection of crop pollen (maize and soybean), and that the greatest pesticide toxicity from the pyrethroid pesticide phenothrin was at the end of the season, associated with two pollen types, one unknown and the other an unidentified species in the Asteraceae, and was likely from insecticide treatments for mosquito control. McArt et al. (2017), studying recently stored bee bread in honey bee colonies pollinating apple,

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Table 4. Partition of *Spiraea* pollen, insecticides, and measures of acute toxicity in a trapped pollen sample (Nursery C, Hive C, 17 August 2015) sorted by color (ND = not detected, ppb = parts per billion)

Color category	Pantone number (TPX)	Sorted weight (g)	% *Spiraea* pollen by weight	Thiamethoxam (ppb)	Clothianidin (ppb)	Acephate (ppb)	Methamidophos (ppb)	Pollen Hazard Quotient	Estimated % of honey bee LD_{50}
Bulk sample		20.96	12.9%	305	31	94	40	70,472	70.5%
Sorted colors									
Mahogany rose	15–1511	0.24	93.5%	680	143	369	241	179,826	180%
Warm sand	15–1214	1.61	74.9	703	202	581	241	202,064	202
Almond buff	14–1116	6.52	19.3	472	79	105	81	117,842	118
Mustard gold	16–1133	0.43	0.46	15	ND	ND	ND	3,583	3.6
Straw	13–0922	1.29	0.15	8	ND	ND	4.7	1,627	1.6
Grape Leaf	19–0511	0.45	0.09	6.8	ND	ND	ND	1,361	1.4
Sunflower	16–1054	6.68	0.08	14	ND	ND	3.1	2,816	2.8
Freesia	14–0852	0.43	0.04	ND	ND	ND	ND	0.23	0
Butterscotch	15–1147	1.48	0.02	8.3	ND	ND	3.8	1,681	1.7
Yolk yellow	14–0846	1.79	ND	6.1	ND	ND	ND	1,221	1.2
Cathay spice	18–0950	0.04	ND	ND	ND	ND	ND	1.27	0

found that while total fungicides were associated with a higher proportion of apple pollen, total insecticides and Pollen Hazard Quotient were not, and were instead associated with the number of different pollen types, and that 85–93% of the pesticide risk was not accounted for by pesticide sprays on apple or drift into the field margins, but came from unidentified sources.

These results point to the need for more detailed analyses in order to identify plant sources associated with high concentrations of insecticides. Our results in this study and in a previous study (Stoner and Eitzer 2013), and the detailed study by Böhme et al. (2018) of daily pollen samples, some of which were sorted into plant fractions, show that pesticide residues in the pollen pool are highly heterogeneous in time and by plant source. Böhme et al. (2018), sorting pollen samples trapped over a single day, and with much lower bee toxicity than in this study, predominantly from fungicides, found that color-sorting was an effective method of concentrating pesticide residue into a subsample (up to 1,600× in one case) where the dominant pollen type could be identified through palynology. In some of their subsamples, the increased concentrations of fungicides were associated with a crop genus such as *Brassica* sp. or *Vitis vinifera*, but in others were associated with a weed genus such as *Achillea* (Böhme et al. 2018).

While Long and Krupke (2016) and McArt et al. (2017) combined pesticide analysis and palynology to show that most of the acute pesticide toxicity in pollen was coming from sources other than the focus crop, and Böhme et al. (2018) also showed that color-sorting could concentrate pesticides and associate them with a plant genus, this study is the first to use the combination of color-sorting, pesticide residue testing, and palynology to identify a single plant genus as a source of highly toxic pollen collected by bees. The residues of thiamethoxam, clothianidin, acephate, and methamidophos found in the sorted 'mahogany rose' pollen, which was 93.5% *Spiraea* in the 17 August sample, were 680, 143, 369, and 241 ppb, respectively (Table 7). Although this was not pure *Spiraea* pollen, the combined concentrations of pesticides highly toxic to bees and the close association with *Spiraea* pollen allow us to conclude that direct consumption of this pollen would have posed an extremely high risk of acute toxicity to any bee.

These samples were chosen for further analysis because of the series of high toxicity samples at Nursery C in August 2015, so they do not allow us to generalize beyond the specifics of that nursery and plant genus at that time. Broader application of the methods used here could assist in discovering the particular combinations of plant, pesticide, and application method that result in high pesticide residues in pollen. Further direct experimentation with *Spiraea* could determine why all four systemic insecticides were found in such high concentrations in only this plant genus, when both thiamethoxam and acephate were used on a wide range of nursery plants at Nursery C from May through August (unpublished pesticide records). Our methods could be improved, now that we know that pollen pellets are often dominated by a single plant genus, but sorted colors are often a mix of genera (as shown in Table 7). In the future, rather than processing and analyzing pollen from individual pellets, we will be dividing up the available supply of color-sorted pollen carefully between the amount needed for pesticide analysis and a number of pellets that will give a more accurate mean composition of pollen species. There is a trade-off, because as the amount of pollen available for pesticide testing drops below 1 g, the accuracy of the pesticide analysis decreases, too, with the methods we are using. There is considerable careful labor required for color-sorting, and the resulting multiplicity of samples also requires expense and time for analysis—both pesticide analysis and pollen identification (either by palynology

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Table 5. Pearson product–moment correlation coefficients and *P*-values for the relationships among pesticides and *Spiraea* pollen in a trapped pollen sample (Nursery C, Hive C, 17 August 2015)

	Clothiandin		Acephate		Methamidophos		*Spiraea*	
Insecticide	*R*	*P*-value	*R*	*P*-value	*R*	*P*-value	*R*	*P*-value
Thiamethoxam	0.974	4×10^{-7}	0.922	5×10^{-5}	0.969	9×10^{-7}	0.938	2×10^{-6}
Clothianidin			0.983	5×10^{-8}	0.978	2×10^{-7}	0.939	2×10^{-5}
Acephate					0.967	1×10^{-6}	0.933	3×10^{-5}
Methamidophos							0.989	8×10^{-9}

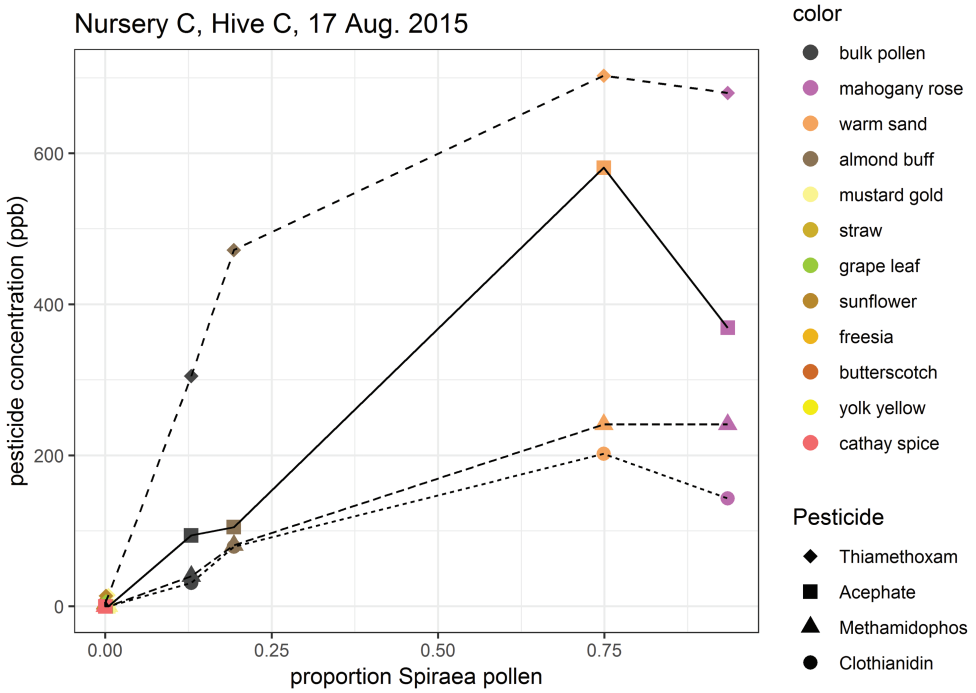


Fig. 2. Relationship between proportion of *Spiraea* pollen and pesticide concentration in a single sample of bulk trapped pollen and in subsamples sorted by color. Note that aside from the bulk pollen and the sorted colors mahogany rose, warm sand, and almond buff, the remaining eight pollen colors are all partially or completely overlapping at or near zero for *Spiraea* pollen and at relatively low concentrations of the four pesticides.

Table 6. Partition of *Spiraea* pollen, insecticides, and measures of acute toxicity in a trapped pollen sample (Nursery C, Hive C, 24 August 2015) sorted by color (ND = not detected, ppb = parts per billion)

Color category	Pantone number (TPX)	Sorted weight	% *Spiraea* pollen (by weight)	Thiamethoxam (ppb)	Clothianidin (ppb)	Pollen Hazard Quotient	Estimated % of honey bee LD_{50}
Bulk sample		8.2497	3.7%	7.8	15	6132	6.1%
Sorted colors:							
Mahogany rose	15–1511	0.3176	94.1%	89	221	80,965	81%
Almond buff	14–1116	1.4863	0.3	6	8.6	3657	3.7
Cumin	18–0939	1.1317	0.04	ND	6.3	1800	1.8
Yolk yellow	14–0846	1.4575	0.004	ND	ND	0.022	0
Straw	13–0922	1.8276	ND	ND	2.3	658	0.7
Sunflower	16–1054	1.1822	ND	6.4	ND	1280	1.3
Grape leaf	19–0511	0.1103	ND	ND	ND	0	0
Freesia	14–0852	0.6706	ND	ND	ND	0	0

or by molecular barcoding). Future studies could also be improved by using molecular barcoding to confirm pollen identification. We currently have a collaborator working out the methods for using molecular barcoding with the overall nursery pollen collection, but we have not used that method here because some of the critical amples have been consumed by the analyses already performed.

This study focussed on measuring exposure through pollen and was not designed to measure effects on the health of the honey bee colonies used for trapping pollen in the nurseries. The question inevitably arises, however—how would exposure to pesticides at these concentrations affect the health of the bees? At the heart of the current controversy over the effects of neonicotinoid insecticides on

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Table 7. Palynological analysis and color-sorted categories for individual pollen pellets trapped at Nursery C in August 2015. Mean % purity was by count of pollen grains, not by weight.

Dominant Pollen Type	No. of pellets	Mean % purity of each pellet	No. of color-sorted categories
Nursery C, Hive C, 17 Aug. 2015			
Spiraea	19	99.3	3
Rhus	10	98.5	3
Lotus corniculatus	9	98.6	2
Nymphaea	6	99.4	3
Solidago	5	99.5	2
Lythrum	5	97.8	1
Trifolium pratense	5	97.6	3
Viola tricolor	5	98.1	2
Ambrosia/Xanthium	4	98.5	2
Eupatorium	2	98.5	1
Robinia	2	96.0	1
Zea mays	2	70.0	1
Buddleja	1	93.0	1
Trifolium repens	1	100.0	1
All pellets analyzed	76	97.9	11
Nursery C, Hive C, 24 Aug. 2015			
Nymphaea	10	98.6	3
Solidago	6	98.1	3
Spiraea	5	98.4	1
Hydrangea	3	97.3	1
Lythrum	3	98.9	1
Rhus	3	97.8	2
Amaranthus	2	94.0	1
Brassica	2	95.7	1
Ajuga reptans	1	97.1	1
Aster novae-angliae	1	99.8	1
Buddleja davidii	1	92.4	1
Centaurea stoebe	1	93.7	1
Polygonatum	1	95.0	1
Zea mays	1	90.4	1
All pellets analyzed	40	97.5	8

bees has been the question of whether 'field realistic' concentrations of these insecticides are high enough, and duration of exposure long enough to cause substantial harm to bee health (Blacquiere et al. 2012, Godfray et al. 2014, Sanchez-Bayo and Goka 2014, Godfray et al. 2015). An additional question is, if we consider trapping honey bee pollen as a method of sampling the available pollen pool, how would other bee species be affected by the pesticide residues found here?

When reviews consider a field-realistic level of neonicotinoid exposure to be 2–6 ppb (Godfray et al. 2014, Godfray et al. 2015), this reflects a consensus concentration of pesticide residues over many studies of pollen and nectar over many studies of seed-treated plants. Our samples from Nurseries M and P would fit into that range, although Nursery C was higher due to multiple samples with higher concentrations during August (Tables 2 and 3). Experimenters measuring the effects of neonicotinoids (Sandrock et al. 2014a) or typical mixtures of insecticides and fungicides (Prado et al. 2018) in pollen on honey bee colony health have fed pollen treated with concentrations of pesticides based on those found in long-term field studies (e.g., 3× the mean concentration found over a 1 yr field study in Prado et al. 2018), and have found subtle effects on colony health, such as increased rates of queen supersedure (Sandrock et al. 2014a), or delayed and less efficient foraging (Prado et al. 2018).

As Sponsler and Johnson (2017) discuss, individual honey bees within a colony are also likely to have highly heterogeneous exposures to pesticide residues, particularly with respect to pesticide residues in pollen. While incoming nectar is widely shared through trophallaxis throughout the colony before being consumed or stored, most incoming pollen is immediately unloaded into a storage cell, along with other pollen loads arriving at the same time, and then packed with honey and saliva to ferment as bee bread (Sponsler and Johnson 2017). This pollen is then consumed primarily by nurse bees, young workers between the ages of 4 and 9 d old, who then convert it into glandular secretions fed to brood and queens, and to a lesser extent to other workers (Sponsler and Johnson 2017). Thus, the effects of heterogeneous pesticide residues in pollen might be more accurately modeled than at present by measuring effects of higher concentrations on young worker bees and then modeling the results for colony function.

Because of the size and complexity of the honey bee 'superorganism,' honey bee colonies may have more mechanisms for mitigating the effects of pesticide exposure and be more resilient than most other bee species (Henry et al. 2015, Straub et al. 2015). Many studies of bumble bee colony growth have found significant effects of nitroguanidine neonicotinoids at concentrations below 10 ppb (Stoner 2016). Sandrock et al. (2014b) demonstrated a 50% reduction in reproduction of the solitary bee *Osmia bicornis* L. at a nectar concentration of 2.87 µg/kg for thiamethoxam and 0.45 µg/kg for clothianidin, generally considered field-realistic concentrations. A direct field comparison showing differences in pesticide effects on honey bees, the bumble bee *Bombus terrestris* L., and *O. bicornis*, pairing oil seed rape fields seeded with clothianidin and cyfluthrin with untreated fields, found no effects on the honey bee colonies, significant effects on colony growth and reproduction for *B. terrestris,* and complete elimination of nesting for *O. bicornis* in the treated sites (Rundlöf et al. 2015).

An example of a bee species that could be more likely than the honey bee to be affected by the concentrations of highly toxic insecticides found in *Spiraea* pollen is *Andrena crataegi* Robertson. *A.crataegi* is a communally nesting bee, actively foraging for only 6 wk in the summer (Osgood 1989), and frequently collected from *Spiraea*, although also using a range of other species (Ascher et al. 2018). The Discover Life website (Ascher et al. 2018) lists 41 species of bees collected from *Spiraea*, including 13 species of *Andrena*, 3 species of *Hylaeus*, 2 species of *Halictus*, 9 species of *Lasioglossum*, 4 species of *Bombus*, and 1 species of *Osmia*. The bee species using *Spiraea* are a heterogeneous group, including both solitary and eusocial species, and varying in size and thus flight range (Greenleaf et al. 2007). The effects of *Spiraea* pollen with a highly toxic concentration of pesticide residues on these bees would also be expected to be highly variable, depending on the specific biology of each bee species.

Although this study found mean concentrations of nitroguanidine neonicotinoids and other insecticides in the same range as previous pollen trapping surveys in Connecticut, we also found, by examining our few highly toxic samples in detail, that much higher concentrations could be obscured within the mixture of diverse pollen sources collected over a week by honey bee colonies. This heterogeneity should be further explored, to the extent resources are available, in order to fully understand the potential for exposure of and effects of these pesticides on honey bees and other bee species.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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