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Potential Exposure of Pollinators to Neonicotinoid Insecticides from the Use of Insecticide Seed Treatments in the Mid-Southern United States

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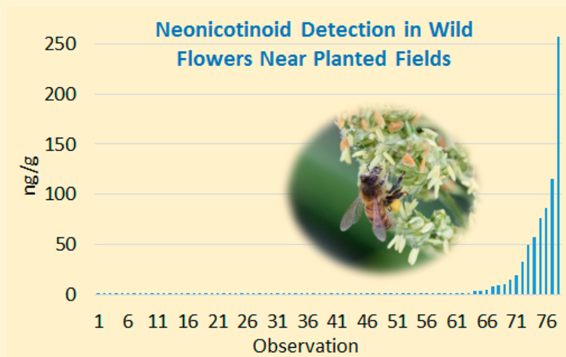
Supporting Information

ABSTRACT: Research was done during 2012 to evaluate the potential exposure of pollinators to neonicotinoid insecticides used as seed treatments on corn, cotton, and soybean. Samples were collected from small plot evaluations of seed treatments and from commercial fields in agricultural production areas in Arkansas, Mississippi, and Tennessee. In total, 560 samples were analyzed for concentrations of clothianidin, imidacloprid, thiamethoxam, and their metabolites. These included pollen from corn and cotton, nectar from cotton, flowers from soybean, honey bees, *Apis mellifera* L., and pollen carried by foragers returning to hives, preplanting and in-season soil samples, and wild flowers adjacent to recently planted fields. Neonicotinoid insecticides were detected at a level of 1 ng/g or above in 23% of wild flower samples around recently planted fields, with an average detection level of about 10 ng/g. We detected neonicotinoid insecticides in the soil of production fields prior to planting at an average concentration of about 10 ng/g, and over 80% of the samples having some insecticide present. Only 5% of foraging honey bees tested positive for the presence of neonicotinoid insecticides, and there was only one trace detection (< 1 ng/g) in pollen being carried by those bees. Soybean flowers, cotton pollen, and cotton nectar contained little or no neonicotinoids resulting from insecticide seed treatments. Average levels of neonicotinoid insecticides in corn pollen ranged from less than 1 to 6 ng/g. The highest neonicotinoid concentrations were found in soil collected during early flowering from insecticide seed treatment trials. However, these levels were generally not well correlated with neonicotinoid concentrations in flowers, pollen, or nectar. Concentrations in flowering structures were well below defined levels of concern thought to cause acute mortality in honey bees. The potential implications of our findings are discussed.



INTRODUCTION

The honey bee, *Apis mellifera* L., is an important pollinator of agricultural crops and utilized for the production of honey by commercial and hobby beekeepers.[1] In Europe, the production of 84% of crop species depends at least to some extent upon animal pollination.[2] The health of pollinators and honey bees in particular continues to receive much attention. The recent decline in honey bee hive populations has been characterized as colony collapse disorder (CCD) by the USDA CCD Steering Committee.[3] CCD is recognized by the rapid loss of adult bees in the colony and is acknowledged by the committee to result from multiple factors.[3,4] Preliminary estimates indicated that 31.1% of managed honey bee colonies in the United States were lost during the winter of 2012−2013.[5] This was a 9% increase compared with the previous winter but similar to a six-year average loss of 30.5%.[6]

The utilization of wild and domesticated bees for pollination is an important part of agriculture in the Mid-South, although not specifically for field crops. However, crop production is also dependent on the use of insecticides to control important pests. Crop preferences by honey bees influence their exposure to pesticides. In the midsouthern region of the US, including Arkansas, Louisiana, Mississippi, west Tennessee, and southern

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Missouri, the major field crops produced are cotton, corn, soybean, rice, and wheat. Corn, cotton, and soybean are visited by pollinators. Corn is less attractive to honey bees than soybean or cotton (A. Cathcot, unpublished), but corn can sometimes be an important pollen source during anthesis.[7]

Multiple mechanisms have been implicated in causing the decline in managed pollinator populations.[3] These mortality factors include *Nosema* microspores, viral pathogens, varroa mites (*Varroa destructor* Anderson and Trueman), tracheal mites (*Acarpis* spp.), small hive beetles (*Aethina tumida* [SHB]), and an insufficient food supply. Recent investigations have suggested that neonicotinoid insecticides may contribute to CCD incidence. Insecticides have been the target of many studies involving collection of bees, bee-collected pollen, and wax. One study reported 121 different pesticides in samples from 887 wax, pollen, bee, and associated hive samples.[8] Almost 60% of the 259 wax and 350 pollen samples contained at least one systemic pesticide, and over 47% had fluvalinate and coumaphos, commonly used as in-hive acaricides, and chlorothalonil, a widely used fungicide. No neonicotinoid residues were found in bees, while 1 thiamethoxam, 11 acetmiprid, 23 thiacloprid, and 14 imidacloprid detections were found from pollen and wax. They concluded that, while exposure to some neurotoxicants may elicit acute and sublethal reductions in honey bee fitness, the effects of these materials in combinations and their direct association with CCD or declining bee health remains to be determined.

Neonicotinoid insecticides are classified as highly toxic to honey bees, although field use rates of neonicotinoids are often proportionally lower than those of insecticides that have less toxicity. Hardstone and Scott reported that honey bees had similar sensitivity to multiple classes of insecticides compared with other insects, although they tended to be more sensitive than other insects to neonicotinoids.[9] Their review found dermal LD50 values for honey bees to imidacloprid and thiamethoxam ranged considerably from 0.13−0.75 and 0.07−0.30 μg per g, respectively. LD50 values for neonicotinoid insecticides when the dose is administered via oral gavage range from about 0.004−0.005 μg per bee for imidacloprid, clothianidin, and thiamethoxam.[10] In contrast, dermal LD50 for bees topically exposed to these same insecticides values are approximately 5−20-fold higher.[10]

The use of neonicotinoid insecticides near bee hives has caused concern. Krupke et al. investigated the possible exposure via planter exhaust contamination of wild flowers and by collection of corn pollen by foraging bees.[11] Dandelion samples were found to contain residues of thiamethoxam and clothianidin. Another study previously indicated that off-target movement of imidacloprid occurred while planting treated corn seed.[12] Samples collected from hives placed near seed-treated fields demonstrated that both thiamethoxam and clothianidin were present in corn pollen, suggesting neonicotinoid seed treatments persisted long enough to be present in pollen. Krupke also reported that dead bees found near hive entrances contained clothianidin.[11] These findings clarified some of the mechanisms by which pollinators could be exposed to agricultural pesticides used as seed treatments.

Yang et al. concluded that sublethal dosages of imidacloprid were able to affect foraging behavior of honey bees.[13] In contrast, Cutler and Scott-Dupree found that honey bee colonies were unaffected by clothianidin on seed-treated canola.[14] Their study indicated no differences in bee mortality, worker longevity, or brood development between control and treatment groups. A study of 16 apiaries found that bee mortality rates were inversely correlated with the number of maize fields treated with imidacloprid, suggesting that this pesticide did not interact with bee fitness.[15] It was concluded that imidacloprid-treated seed in maize had no negative impact on honey bees. Tasei et al. concluded that the bumblebee (*Bombus terrestis* L.) foraging, homing behavior, or colony development were not significantly affected by sublethal exposure to neonicotinoid insecticides.[16]

The impact of neonicotinoid on pollinators and specifically honey bees has been studied across a broad geography by many scientists. The contribution of neonicotinoids and pesticides in general to CCD remains highly controversial. Our objective was to provide additional information about the potential routes and levels of exposure of pollinators to neonicotinoid insecticides in an intensive crop production area of the Mid-South.

■ MATERIALS AND METHODS

Research was done during 2012 to evaluate the potential exposure of pollinators to neonicotinoid insecticides used as seed treatments on corn, cotton, and soybean. In total, 560 samples were analyzed for concentrations of clothianidin, imidacloprid, thiamethoxam, and their metabolites. These included pollen from corn and cotton, nectar from cotton, flowers from soybean, honey bees and pollen carried by foragers returning to hives, preplanting and in-season soil samples, and wild flowers adjacent to recently planted fields of cotton, corn, or soybean.

Sampling Locations and Processing. Many samples collected in this study were from production fields that were representative of cropping systems in the Mid-South. Fields were located in intensive agricultural production areas of Arkansas, Mississippi, and west Tennessee. Other samples were collected from replicated trials of various insecticide seed treatments in cotton, corn, and soybean with uniform insecticide seed treatment rates. A summary of the kinds and methods of sample collection is presented below. Additional details are provided in the Supporting Information (SI).

(1) Pre-Plant Soil Samples. Prior to planting in each state, soil was collected from production fields with a known use of insecticide seed treatment during the previous cropping system. Fields included cotton (12), corn (8), soybean (7), and wheat (1).

(2) Post-Planting Samples of Wild Flowers. Wild flowers, representing potential sources of pollen and nectar for pollinators that could be exposed to windblown talc that was contaminated with neonicotinoids, were collected adjacent to 49 production fields of cotton, corn, or soybean that had been planted during the previous week. Flowers were collected from four different sides of each field whenever possible, but these samples were often composited to increase assay sensitivity and reduce costs. The species of wild flowers collected varied considerably and depended upon what flowers were available. The average sample distance from field edges was 20 m.

(3) Samples of Bees and Bee Pollen. Foraging honey bees and the pollen they carried were collected from commercial apiaries. A total of 15 apiaries and 4 hives per apiary were sampled. Apiaries were located, on average, approximately 180 m from agricultural fields. We typically collected samples at two different times of the

Table 1. Levels of Neonicotinoid Insecticides (Mean ± Standard Deviation), Total Detections ≥1 ng/g, and Percent Detections Greater than or Equal to 1 ng/g for Wild Flowers Collected Adjacent to Recently Planted Fields of Cotton, Corn, and Soybean, and for Soil Collected Prior to Planting from Agricultural Production Fields

	preseason soil				wild flowers			
	clothianidin	imidacloprid	thiamethoxam	total	clothianidin	imidacloprid	thiamethoxam	total
mean (ng/g)	3.4 ± 5.3	4.0 ± 5.5	2.3 ± 4.7	9.7 ± 8. 8	1.4 ± 7.1	1.1 ± 6.0	7.2 ± 31.9	9.6 ± 34.8
total detections ≥1 ng/g	50	55	54	90	5	5	11	18
% detections ≥1 ng/g	45	49	48	80	6	6	14	23
maximum level detected	26	26	36	39	53	48	256	257
N (samples analyzed)				112				78

season representing the normal planting (April−May) and flowering windows (June 15−September 9). A minimum of ten bees, usually 30 or more, were collected from each hive. In some cases, too few foraging bees were carrying pollen to collect four samples per apiary. Bee pollen samples from each apiary were composited by date, but even then, there were several cases where sample mass did not allow for analysis. Some bee samples were also composited within a location to increase assay sensitivity and reduce costs.

(4) Samples from Tests of Insecticide Seed Treatments. Samples were collected from multiple locations where insecticide seed treatments were being evaluated in corn, cotton, and soybean. Samples were collected from four replicates at each location during early flowering growth stages defined as R1 (early tasseling) in corn, R1 or R2 (early flowering) in soybean, and approximately 10−14 days after flowering had begun in cotton. Additional details are provided below and in the SI.

Soybean flowers were collected from four insecticide seed treatment tests. Treatments were insecticide-free seed and standard insecticide seed treatment rates of thiamethoxam (0.5 g active ingredient (ai) per kg seed, Cruiser 5FS, Syngenta Crop Protection), imidacloprid (0.78 g ai per kg seed, Gaucho 600, Bayer CropScience), and clothianidin (0.5 g ai per kg seed, NipsIt Inside, Valent USA). Samples were composited across replicates by treatment within each of the four locations.

Corn pollen was collected from four trials within 2−4 days of tassel emergence for each of the following seed treatments: no insecticide seed treatment, thiamethoxam (0.25 mg ai per seed, Cruiser 5F, Syngenta Crop Protection), thiamethoxam (0.5 mg ai per seed), clothianidin (0.25 mg ai per seed, Poncho 250, Bayer CropScience), and clothianidin (1.25 mg ai per seed). Corn pollen was composited by treatment across replicates within each of the four locations.

Cotton pollen was collected from five tests. Pollen was collected from untreated seed and seed treated with thiamethoxam (0.375 mg ai per seed, Cruiser Avicta, Syngenta Crop Protection) and imidacloprid (0.375 mg ai per seed, Aeris, Bayer CropScience). At three of the five locations, cotton nectar was collected from the same treatments. Cotton pollen and nectar were also composited by treatment across replicates within each of the five locations.

Soil samples were also collected at the same time as pollen or flower samples. This included samples from all but one cotton test (SI Table S1). Collection methods were similar to those described for preplant soil samples. These samples were not composited across replicates.

Chemical Analyses. Samples were analyzed to determine the levels of neonicotinoid residues by the USDA AMS Science and Technology Laboratory Approval and Testing Division of the National Science Laboratories' Gastonia Lab in Gastonia, NC. This laboratory is accredited to ISO/IEC 17025:2005 for specific tests in the fields of chemistry and microbiology, including testing for pesticide residues. The samples were extracted for analysis of agrochemicals using a refined methodology for the determination of neonicotinoid pesticides and their metabolites using an approach of the official pesticide extraction method (AOAC 2007.01), also known as the QuEChERS method, and analyzed by liquid chromatography coupled with tandem mass spectrometry detection (LC/MS/MS).[17−20] Samples were analyzed for the presence of 17 insecticides or their metabolites (SI Table S4). Quantification was performed using external calibration standards prepared from certified standard reference material.

Only detections of clothianidin, imidacloprid, and thiamethoxam are reported. The method detection limit for these compounds was 1 ng/g (1 ppb). Neonicotinoid metabolites were found only in soil collected during early flowering from insecticide seed treatment trials, and they represented less than 1.5% of total concentration. Other neonicotinoid insecticides were not detected. Where averages are presented, we assumed nondetection was equivalent to 0.0 ng/g. "Trace" detections (nonquantifiable detection <1 ng/g) were also included in the analytical reports, and these values were assumed to be 0.5 ng/g when calculating averages.

RESULTS

Pre-Plant Soil Samples. Neonicotinoid insecticides were commonly found prior to planting in the soil of production fields that were previously planted to cotton, corn, or soybean, indicating residual carry-over of insecticides used as seed treatments or foliar sprays during previous cropping seasons. Across 112 samples representing 28 fields, 80% of samples had a detection level of ≥1 ng/g, averaging approximately 10 ng/g with a median concentration of 8 ng/g (Table 1, SI Figure S1). Clothianidin, imidacloprid, and thiamethoxam were detected in approximately 50% of preseason soil samples. Two or more neonicotinoid insecticides were detected in 43% of the samples. The highest level of detection in any one sample was 39 ng/g from a field previously planted in cotton that was treated with a thiamethoxam seed treatment. However, it should be noted that cotton in the Mid-South is often treated with foliar neonicotinoid insecticides for the control of the tarnished plant bug (*Lygus lineolaris* Palisot De Beauvois) and other pests. Neonicotinoids were detected in soil collected from two of the four fields sampled that were thought not to have been exposed to neonicotinoid insecticides during the previous season.

Post-Planting Samples of Wild Flowers. Approximately 23% of wild flower samples collected around recently planted fields tested positive for neonicotinoid insecticides at a level of 1 ng/g or greater (Table 1, SI Figure S2). The average total neonicotinoid detection level was about 10 ng/g. The greatest level of detection (257 ng/g) was from flowers collected adjacent to a corn field in Tennessee that was planted the previous day with thiamethoxam-treated seed. There was no detection of insecticides in flowers collected within 20 m of this same field. However, two samples collected at 50 and 100 m from the field edge were composited prior to analysis and averaged 256 ng/g of thiamethoxam. Subsequent investigation revealed that one of these samples was collected where the farmer filled the planter with seed. The second highest concentration of neonicotinoid insecticides (115 ng/g) was from wild flower samples within 1−5 m of a field edge that were collected within 2 h of planting. This field was divided and planted with both imidacloprid-treated cotton seed and thiamethoxam-treated soybean on the same day. Both insecticides were detected about equally. These two samples accounted for over 50% of the total neonicotinoid concentration detected across all 78 samples analyzed.

Samples of Bees and Bee Pollen. Of the 74 samples assayed, representing 15 apiaries and 60 hives, neonicotinoid insecticides were only detected at a level of 1 ng/g or greater in two samples of foraging bees collected while returning to the hive. Across all samples, the average total concentration of neonicotinoid insecticides was less than the method detection limit of 1 ng/g. The highest level of detection was for imidacloprid (48 ng/g) from a hive in Fayette Co., TN. Interestingly, neonicotinoids were not detected in bee samples from three other hives within that apiary. These samples were collected during early flowering and appear to reflect exposure from a recent foliar application of imidacloprid made to a cotton field that was within 25 m of the apiary. The other positive sample was clothianidin (≈ 10 ng/g) from bees collected from an apiary in Lowndes Co., MS. This apiary was approximately 500 m from corn and soybean fields. Bee samples at this location were accidentally composited across the early and midseason sampling dates, so it is unclear whether this detection could reflect exposure from seed treatments used at planting or foliar application that might have been made to nearby production fields.

From the same apiaries, neonicotinoids were only detected in 1 of 24 pollen samples collected from returning foragers. Both imidacloprid and thiamethoxam were detected in this sample at trace levels (< 1 ng/g). Thus, the average concentration of neonicotinoids across all samples was well below the method detection limit of 1 ng/g. For the Fayette County location where imidacloprid was detected in bees from one hive, no insecticides were detected in the composited sample of pollen collected from bees at this apiary. There was insufficient pollen available for analysis at the location where clothianidin was detected in bees from the Lowndes Co., MS location.

Samples from Tests of Insecticide Seed Treatments. There was no detection of neonicotinoid insecticides in flowers collected from four soybean tests where neonicotinoid seed treatments were being evaluated. Imidacloprid was not used as a corn insecticide seed treatment nor was it detected in our samples of corn pollen. Thiamethoxam was detected at very low levels in corn pollen, averaging less than the detection limit of <1 ng/g across four experiments where it was included as a seed treatment (Table 2). Clothianidin was detected for treatments of 0.25 and 1.25 mg ai per seed at an average level of about 3 and 6 ng/g, respectively. For unknown reasons, two samples had a much higher concentration of clothianidin (9 and 23 ng/g) than the others, and this accounted for the majority of clothianidin found in corn pollen (SI Figure S3). Clothianidin was also detected at trace levels in plots of untreated corn seed and seed treated with thiamethoxam.

Table 2. Levels of Neonicotinoid Insecticides (Mean ± Standard Deviation), Total Detections ≥1 ng/g, and Percent Detections Greater than or Equal to 1 ng/g for Corn Pollen from Plots Treated with Different Insecticide Seed Treatments

	clothianidin	thiamethoxam	total
total detections ≥1 ng/g	2	1	4
% detections ≥1 ng/g	10	5	20
maximum level detected	23.1	1.0	23.1
Mean by Seed Treatment			
untreated	0.1 ± 0.25	0.1 ± 0.3	0.3 ± 0.3
clothianidin[a]	2.5 ± 4.57	0.1 ± 0.3	2.6 ± 4.5
clothianidin[a]	5.9 ± 11.5	0.0 ± 0.0	5.9 ± 11.5
thiamethoxam[a]	0.0 ± 0.0	0.4 ± 0.3	0.4 ± 0.3
thiamethoxam[a]	0.1 ± 0.3	0.4 ± 0.3	0.5 ± 0.4
N (composited samples)			20

[a]Applied at a rate of 0.25, 1.25, 0.25, and 0.5 mg ai per seed, respectively.

Neonicotinoid insecticides were not detected in cotton nectar. Low concentrations of imidacloprid or thiamethoxam were detected in pollen from cotton treated with insecticide seed treatments. The average total concentration of neonicotinoid insecticides was less than the method detection limit of 1 ng/g. In the 15 composited samples that were analyzed, there was only one sample with a total neonicotinoid concentration greater than 1 ng/g. In this sample, 2.9 and 1.1 ng/g of imidacloprid and thiamethoxam were found, respectively. Trace levels of clothianidin were detected in one sample even though this insecticide was not used in the test.

During early flowering, neonicotinoids were commonly detected in the soil of soybean, corn, and cotton treated with insecticide seed treatments (Tables 3 and 4, SI Figures S4−S6). Across all treatments, 63%, 62%, and 98% of soil samples had a total concentration of neonicotinoid insecticides ≥1 ng/g for soybean, corn, and cotton, respectively. For soybean, soil from plots of imidacloprid- and thiamethoxam-treated seed had an average total neonicotinoid concentration of about 25 ng/g. Soil from plots of clothianidin-treated seed averaged <5 ng/g of clothianidin.

Average detection levels in soil from plots of neonicotinoid-treated corn seed were mostly consistent with the treatment applied (Table 3). The highest average concentration of clothianidin in soil (18 ng/g) was found in plots with seed treated at 1.25 mg ai per kernel of this insecticide. Similarly, soil from thiamethoxam-treated plots averaged 5 and 11 ng/g for treatments of 0.25 and 0.5 mg ai per kernel, respectively. Clothianidin and low levels of imidacloprid were detected in all treatments, including those not treated with insecticide, suggesting the presence of residual neonicotinoid insecticides from the previous cropping season.

Across all samples, the highest concentrations of neonicotinoid insecticides were found in soil collected from cotton plots (Table 4). Soil from plots of untreated cotton seed averaged a total neonicotinoid concentration of almost 25 ng/g, again indicating the presence of residual insecticides from previous cropping seasons that likely would have included foliar application of these same insecticides. Soil from plots of imidacloprid- and thiamethoxam-treated cotton seed had an

Table 3. Levels of Neonicotinoid Insecticides (Mean ± Standard Deviation), Total Detections ≥1 ng/g, and Percent Detections Greater than or Equal to 1 ng/g for Soil Collected from Plots of Soybean and Corn That Were Treated with Different Insecticide Seed Treatments

	soybean soil (4 locations)				corn soil (5 locations)			
	clothianidin	imidacloprid	thiamethoxam	total	clothianidin	imidacloprid	thiamethoxam	total
total detections ≥1 ng/g	26	25	16	40	34	24	34	62
% detections ≥1 ng/g	41	39	25	63	34	24	34	62
maximum level detected	26	84	113	124	113	77	75	116
Mean by Seed Treatment								
untreated	1.5 ± 2.4	0.8 ± 1.4	0.5 ± 1.3	2.8 ± 3.1	3.0 ± 6.8	0.1 ± 0.3	1.0 ± 2.4	4.0 ± 8.4
clothianidin[a]	4.2 ± 6.6	0.4 ± 0.8	0.2 ± 0.7	4.8 ± 6.9	3.6 ± 6.3	0.8 ± 1.6	0.2 ± 0.6	4.6 ± 6.2
clothianidin[b]					18.0 ± 30.1	1.0 ± 2.0	0.1 ± 0.4	19.1 ± 30.1
imidacloprid[c]	2.6 ± 6.6	23.5 ± 25.8	0.3 ± 1.0	26.4 ± 30.0				
thiamethoxam[d]	5.1 ± 6.0	1.2 ± 2.2	17.5 ± 30.0	23.8 ± 33.4	3.6 ± 12.2	1.0 ± 1.8	4.7 ± 6.2	9.3 ± 14.3
thiamethoxam[e]					5.6 ± 10.4	0.8 ± 1.3	11.2 ± 20.2	17.7 ± 29.3
N (samples analyzed)				64				100

[a]Applied at a rate of 0.5 g ai per kg seed for soybean and 0.25 mg ai per seed for corn. [b]Applied at a rate of 1.25 mg ai per seed, respectively. [c]Applied at a rate of 0.78 g ai per kg seed. [d]Applied at a rate of 0.5 g ai per kg seed for soybean and 0.25 mg ai per seed for corn. [e]Applied at a rate of 0.5 mg ai per seed.

Table 4. Levels of Neonicotinoid Insecticides (Mean ± Standard Deviation), Total Detections ≥1 ng/g, and Percent Detections Greater than or Equal to 1 ng/g for Soil Collected from Cotton Plots That Were Treated with Different Insecticide Seed Treatments

	clothianidin	imidacloprid	thiamethoxam	total
total detections ≥1 ng/g	9	45	31	47
% detections ≥1 ng/g	19	94	65	98
maximum level detected	70	362	658	735
mean by seed treatment				
untreated	1.3 ± 3.5	19.7 ± 28.9	3.5 ± 5.0	24.5 ± 31.7
imidacloprid[a]	2.2 ± 4.4	65.4 ± 98.4	1.7 ± 2.0	69.4 ± 102
thiamethoxam[a]	6.5 ± 17.9	20.8 ± 41.6	102 ± 170	129 ± 185
N (samples analyzed)				48

[a]Imidacloprid and thiamethoxam applied at a rate of 0.375 mg ai per seed.

average total neonicotinoid concentration of 69 and 129 ng/g, respectively. There was significant variation in the concentration of neonicotinoid insecticides found in the soil at different trial locations (SI Figure S6). The mean concentrations of imidacloprid in soil from plots of imidacloprid-treated seed were 23, 185, 43, and 26 ng/g for each of the four test locations. Similarly, thiamethoxam levels from plots of thiamethoxam-treated seed were 141, 323, 47, and 6 ng/g for these same locations, respectively. Soil type, water and soil movement to low lying areas, or foliar insecticide applications made the previous cropping season might explain these differences.

In all crops, slightly elevated levels of clothianidin were detected in the soil of plots with thiamethoxam-treated seed. This likely reflects metabolism of thiamethoxam to clothianidin within the soil, a process also reported within insects and plants.[21]

DISCUSSION

There are many examples that demonstrate a sometimes considerable value of crop protection provided by neonicotinoid seed treatments in the Mid-South, especially in cotton, corn, and rice. Foliar insecticide applications typically do not provide adequate protection against seed and seedling pests as do systemic treatments to the seed or in the seed furrow. Alternatives to neonicotinoid insecticides are diminishing and may also have negative safety and environmental profiles. For example, aldicarb (Temik 15G, Bayer CropScience) applied as an in-furrow granular is no longer available as an alternative at-planting treatment in cotton, mostly because of safety concerns owing to its acute toxicity to mammals.

Our study was intended to evaluate the potential routes of pollinator exposure to neonicotinoid insecticides being used ubiquitously as seed treatments in the midsouthern US. Nearly 100% of cotton and corn seed planted in the Mid-South are treated with an insecticide. The standard use rate of imidacloprid or thiamethoxam in cotton is 0.375 mg ai per seed. All major seed corn companies treat seed with either clothianidin or thiamethoxam prior to distribution. The most common use rate of clothianidin and thiamethoxam for corn grown in the Mid-South is 0.25 mg ai per kernel. However, 0.50 mg rates are becoming more commonly adopted. Clothianidin at 1.25 mg ai per kernel is occasionally used, typically in scenarios of expected high pest pressure. Over 70% of soybean seed are also treated with insecticides in the Mid-South, most commonly thiamethoxam or imidacloprid at the rates evaluated in this study. The use of insecticide seed treatments in soybean has increased rapidly in the past five years.[22] Talc or graphite is also commonly used as a seed lubricant in vacuum planters. The emission of contaminated "dust" during planting is perceived as a major route of pollinator exposure to neonicotinoid insecticides.[11]

We detected neonicotinoid insecticides in samples of wild flowers collected shortly after the planting of commercial fields,

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clearly implicating contamination from planter dust. Over 70% of samples had no detectable concentration of neonicotinoid insecticides, but a few samples had much higher concentrations suggesting a mosaic of exposure risks to pollinators foraging on wild flowers (SI Figure S2). It is unclear whether concentrations of neonicotinoid insecticides found in wild flowers are reflective of likely exposure to bees while foraging. It is possible that insecticide concentrations are higher or lower in pollen or nectar being targeted by bees. Regardless, efforts to reduce off-site movement of planter dust can hopefully be implemented to mitigate this potential route of exposure to pollinators. The overall risk of exposure to pollinators from foraging on contaminated wild flowers should be less than suggested by our data because we collected flowers in close proximity to fields, typically within 3 days of planting, and rainfall seldom occurred between planting and sample collection. There is the possibility of chronic exposure of pollinators to exposure from planter dust because multiple crops are planted across a wide range of planting dates in the Mid-South. However, this may reduce the risk of acute exposure. A narrower planting window in more northern crop production regions may partially explain any geographic differences in bee health. Another factor to consider is the intensity of crop production. For example, over 50% of the total land acreage in Iowa, Illinois, Indiana, and Minnesota is planted to principal crops.[23] In contrast, less than 20% of the total land acreage is planted to major field crops in Arkansas, Louisiana, Mississippi, and Tennessee.[23] Thus, there appears to be less potential for exposure to planter dust in the Mid-South, and alternate food sources are almost certainly more common and diverse.

It is encouraging that insecticides were either not detected or found at very low levels in soybean flowers, cotton pollen, and cotton nectar in plots treated with insecticide seed treatments. Thiamethoxam was detected at 1 ng/g or less in all samples of corn pollen where it was used as a seed treatment. For corn treated with clothianidin at 1.25 mg ai per kernel, we found that the average concentration of clothianidin in pollen (Table 2) was similar to that previously reported.[11] Because this highest rate is not widely used in the Mid-South, expected exposure of bees through corn pollen should be less.

The US Environmental Protection Agency, in support of the proposed risk assessment process for bees, has established "levels of concern" for exposure of honey bees to pesticides.[24] Acute levels of concern were defined as 40% of the LD50 value. Based on maximum consumption rates of pollen and nectar for foraging honey bees, insecticides in nectar are far more concerning than those in pollen (Table 5). The levels of concern presented in Table 5 presume that bees from a colony are feeding exclusively on contaminated food sources, but this behavior is conceivable for acute exposure, for example where managed bees are used for pollination in crops. Regardless, the concentrations of neonicotinoid insecticides we found in soybean flowers, corn and cotton pollen, and cotton nectar were well below acute levels of concern, even for more sensitive life stages of honey bees.

Table 5. Approximate Acute Levels of Concern (LOC) for Neonicotinoid Insecticides in Food Sources of Honey Bees Based on Adult Worker Consumption

food source	LD50 (μg per bee)[a]	EPA safety factor[b]	consumption (mg)[b]	LOC (ng/g or ppb)
pollen	0.004	0.4	9.5	168
nectar	0.004	0.4	292	5.5

[a]Representative LD50 values for clothianidin, imidacloprid, or thiamethoxam. [b]EPA defined risk quotient (RQ) and maximum daily consumption.[22]

The presented levels of concern do not reflect potential impact from chronic exposure or impact on other pollinators. The risk of chronic pesticide exposure to honey bees or other pollinators is best assessed through relatively long-term exposure of sublethal doses via feeding studies. The EPA suggests developing chronic risk quotients (RQ) using "no observable adverse effect concentrations (NOAEC)" once acceptable toxicity test designs are available. Some long-term feeding studies with honey bees indicate minimal or no effects from chronic exposure to imidacloprid up to a concentration of 20 ng/g when delivered in fortified honey.[25,26] In contrast, Whitehorn et al. reported that lower, field-realistic doses fed in pollen or sugar water reduced bumble bee (*Bombus terrestris* L.) colony growth and queen production.[27] The neonicotinoid residues we found in corn pollen were similar to the median concentrations found by Pilling et al.[28] For treated corn seed, they found median concentrations of thiamethoxam and clothianidin ranged from 1 to 7 ng/g in pollen collected from honey bees foraging on corn. Median residues of these same insecticides in the pollen and nectar of oilseed rape were below the limits of detection (1 ng/g). Hive health was not affected by long-term exposure to corn or oilseed rape treated with neonicotinoid seed treatments. Similarly, Pohorecka et al. found that even higher concentrations of clothianidin in pollen loads had no effect on the development and long-term survival of honey bee colonies, in part because corn pollen reflected a low proportion of the total pollen collected by bees.[29]

With two exceptions, neonicotinoid concentrations in foraging bees collected from apiaries within these intensive agricultural areas were too low to be detected, and there was no detection of insecticides in the pollen from these bees. Foliar applications of neonicotinoid insecticides, especially imidacloprid and thiamethoxam, are commonly applied to cotton and occasionally to soybean in the Mid-South. Because these applications may occur near or during bloom, this appears to be a more likely route of exposure to pollinators. Dively and Kamel reported considerably higher concentrations of neonicotinoids in the pollen and nectar of pumpkins when foliar-applied or chemicated insecticides were applied during flowering compared with at-planting applications.[30] Current research is addressing the potential exposure to pollinators resulting from foliar applications to field crops grown in the Mid-South.

Depending upon the crop and seeding rate, the amount of active neonicotinoid insecticide used on seed typically varies from 20 to 100 g ai per ha for corn treated with clothianidin at 1.25 mg ai per seed treatment. This would equate to applied concentrations of about 18−90 ng/g in the upper 7.6 cm of the entire soil profile, where seed are planted and our in-season soil samples were collected. However, we collected our soil within ±7.6 cm of the seed furrow. Expected concentrations at the time of planting in that more limited soil profile would equate to about 113−565 ng/g. Our detection levels in soil during early flowering were considerably lower, particularly in corn and soybean (Tables 3 and 4, SI Figures S4−S6). Besides uptake by the plants, this reduction could reflect degradation, leaching, or erosion away from the furrow.[31−33] Regardless, our data indicate that neonicotinoid insecticides may persist in the soil at low levels for at least one year. Neonicotinoids were detected in soil prior to planting (Table 1, SI Figure S1), although at considerably lower levels than those collected during early flowering in crops treated with insecticide seed treatments. The persistence of clothianidin, imidacloprid, and thiamethoxam resulting from soil applications is well documented. Hopwood et al.'s review reported half-lives in aerobic soil ranging from 25 to 1155 days depending upon the chemical and test conditions, with thiamethoxam having a notably shorter half-life than clothianidin or imidacloprid.[34] It should be considered that pesticide detection in wild flowers near the perimeter of fields could result from movement of contaminated water[35] or soil from nearby cropping areas.

The methods used to process soil samples would likely extract pesticides that would otherwise be unavailable for plant uptake (J. Barber, personal comm.), and it is unclear whether insecticide concentrations in the soil would directly relate to concentrations in plants. Neonicotinoid insecticides were generally not detectable or at much lower concentrations in pollen or nectar than were observed in the soil. However, our data suggest that clothianidin levels in corn pollen were somewhat relatable to concentrations in the soil. For example, an average of 18 ng/g clothianidin was detected in the soil of plots treated at 1.25 mg ai per seed, and 6 ng/g was detected in the pollen of corn collected at the same time (Tables 2 and 3). Concentrations in soil and pollen for corn treated with clothianidin at 0.25 mg ai per seed were about 4 and 3 ng/g, respectively. The relatively low levels of neonicotinoid insecticide we found in pollen and nectar may partially result from the dilution of insecticide available in the soil within a rapidly growing plant.

Results of these surveys indicate that there is a potential risk of pollinator exposure to neonicotinoid insecticides used as seed treatments in corn, soybean, and cotton in areas of intensive agricultural production. However, no information exists that suggest the typical levels of neonicotinoid insecticides detected in this study pose a serious risk to the health of honey bees. Additional research is underway to quantify how quickly these insecticides are broken down within plant tissues and soil over time. Research is also needed to determine if and how pollinators are exposed to neonicotinoid insecticides through the pathways examined in these studies, and moreover, to understand how the levels of neonicotinoids detected in wildflowers and crop pollen affect pollinator health. Finally, these results do not define the risk of neonicotinoid insecticides to pollinator health because we have not measured actual exposure or toxicity at the levels identified. However, based on the low percentage of detections in honey bees returning to hives and the pollen they were carrying, as well as the generally low levels found in pollen and nondetectable concentrations in soybean flowers and cotton nectar, it appears that the overall contribution of seed-applied neonicotinoid insecticides to declining colony health is relatively low in the midsouthern US.

■ ASSOCIATED CONTENT

Supporting Information

Additional tables and figures as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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All authors have given approval to the final version of the manuscript.

Notes

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