

Article

Temporal Profile of Neonicotinoid Concentrations in Cotton, Corn, and Soybean Resulting from Insecticidal Seed Treatments

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Abstract: Neonicotinoids have been implicated as a contributing factor to the observed decreases in honey bee populations. It has been suggested that honey bees can be exposed to seed-treated neonicotinoids through pollen and nectar from treated plants. To investigate the uptake and persistence of neonicotinoids in plant tissue and soil, we conducted seed treatment trials with corn, cotton, and soybean planted in Mississippi, Arkansas, and Tennessee during the 2013 and 2014 growing seasons. Leaf tissue was collected and analyzed beginning shortly after emergence until plants began to flower to better understand how neonicotinoid concentrations change in plant tissues over time. The youngest leaf in the terminal of the plant was sampled as an indicator of the neonicotinoid concentrations within the plant. Soil samples were also collected and analyzed for neonicotinoid concentrations at the first and last sampling dates. The mean clothianidin concentrations in corn treated with Poncho[®] 250, 500, and 1250 seed treatments declined by 99.3, 99.3, and 97.8 percent, respectively, as the plants developed from seedlings to reproductive plants. The mean concentration of imidacloprid detected in Aeris[®]-treated cotton decreased by 99.6 percent during the sampling period. For cotton seed treated with Avicta[®] Duo, the mean concentrations of thiamethoxam and clothianidin in leaf tissue declined by 99.9 and 100 percent, respectively, by the time flowering occurred. There was a 99.9 percent reduction in the mean concentration of thiamethoxam by the time of flowering in leaf tissue from soybean treated with a CruiserMaxx[®] seed treatment. Mean clothianidin concentrations completely diminished (<1 ng/g) in CruiserMaxx[®]- and Poncho[®]/VOTiVO[®]-treated soybean plants by the time plants reached reproductive growth. The data for neonicotinoid concentrations in the soil were more variable than leaf tissue samples, and the reduction in neonicotinoid concentrations in leaf tissues did not closely correlate with concentrations in the soil. Our results suggest that neonicotinoid insecticides, when used as seed treatments in these crops, decline rapidly throughout vegetative growth stages. However, the biological impact on target or non-target arthropods was not examined.

Keywords: seed treatment; neonicotinoid; honey bee

1. Introduction

The neonicotinoids are a widely employed class of insecticides used in row crop agriculture to protect against a broad range of economically important pests including piercing and sucking insects as well as some below-ground coleopteran and lepidopteran pest species [1]. Since the introduction of the first neonicotinoid insecticide in 1991 [1],

usage of this insecticide class has dramatically increased across the United States [2]. This class of chemicals has been rapidly adopted because of several key attributes including their systemic nature and persistence, ease of application, low mammalian toxicity, and efficacy against target pests resistant to other insecticide classes [3]. In the United States, one of the primary uses of neonicotinoids in agriculture is as a seed treatment that is fixed to the outer coating of crop seed prior to planting [3]. This prophylactic approach serves more as a preventative treatment for potential pest problems rather than a traditional integrated pest management tactic [2]. Neonicotinoid seed treatments are one of the few control options available to prevent yield and economic losses from many early-season pests [4].

Almost all corn seed planted in the United States are currently treated with a neonicotinoid seed treatment before planting to protect corn seedlings from many below-ground and early-season pests [5,6]. For most of the corn in the United States, neonicotinoids provide protection against wireworms and corn rootworm species [6]. These pests feed on the primary and secondary roots, which can lead to instability and lodging of the affected corn plants [7]. Most cotton seed are commonly treated with neonicotinoids for protection against thrips and below-ground pests that attack the roots [8]. Thrips have the most impact on cotton plants from emergence until the four to five true-leaf stage [9]. Many soybean growers treat seed with neonicotinoids to prevent stand loss and replanting caused by early-season insect pests [10]. In Midsouthern U.S. corn, cotton and soybean production systems, neonicotinoid seed treatments provide yield and economic increases when compared to untreated seed in experimental trials [6,8,10].

The killing efficiency and widespread use of these products as well as the systemic nature and persistence of insecticidal activity have led to concerns of potential impacts on non-target arthropods [3]. Neonicotinoid seed treatments have been implicated as a potential contributor to recent honey bee, *Apis mellifera* (L.), colony losses [11]. Feeding experiments when colonies are continuously fed sub-lethal doses of neonicotinoids have resulted in adverse effects to honey bee behaviors essential to foraging success [12–14], although some consider that the continuous feeding of sub-lethal doses does not accurately represent field scenarios [11]. Honey bees of the same colony forage on different floral sources simultaneously [15], most of which are not likely contaminated with neonicotinoids, thus diluting the levels of neonicotinoids within the stored food of a colony [15]. Nectar and honey entering the hive undergo further processing, storage, and exposure conditions [16] that can cause rapid degradation of neonicotinoid compounds within the stored food [15]. Regardless of opinions on exposure level, the evidence is clear that honey bees do come into contact with neonicotinoid compounds. Neonicotinoid compounds have been detected in honey bee hive wax and collected pollen [17–19].

Honey bees can be exposed to neonicotinoid seed treatment compounds through multiple routes. Contaminated pollen and nectar can be collected from multiple treated crops as well as from neighboring flowering vegetation near treated fields. Wildflowers near field borders can test positive for neonicotinoids as a result of drifting seed treatment particles in the exhaust that exits vacuum-driven planters [4,17]. Flowering vegetation may absorb neonicotinoids from soil in fields planted with neonicotinoid dressed seeds [4,17,20,21], but some of these soil-bound compounds potentially may not be available for plant uptake [21]. Floral structures of treated crops make for a logical source of neonicotinoid interactions with foraging honey bees.

Neonicotinoid compounds have been detected in corn pollen from plants treated with neonicotinoid seed treatments [4,17,21,22]. During anthesis, the pollen, and stems/leaves of corn plants treated with an imidacloprid seed treatment contained average imidacloprid concentrations of 2.1 and 4.1 ng/g, respectively [22]. Pollen from corn plants seed treated with the highest labeled rate of clothianidin contained average clothianidin concentrations of 3.9 ng/g and thiamethoxam concentrations of 1.7 ng/g [17]. Corn pollen from plants treated with clothianidin seed treatments of 0.25 and 1.25 mg per seed contained concentrations of clothianidin of 3 and 6 ng/g, respectively [4].

Cotton and soybean nectar and pollen have also been evaluated for neonicotinoid concentrations. Cotton pollen and nectar from imidacloprid seed-treated plants and soybean flowers from thiamethoxam seed-treated plants were tested and found to contain little to no neonicotinoid concentrations [4]. To better understand how initial neonicotinoid concentrations from seed treatments diminish between planting and reproductive growth, multiple experiments were conducted in the Midsouthern United States in corn, cotton, and soybean.

2. Materials and Methods

Multiple experiments were conducted at agricultural research stations in Mississippi, Arkansas, and Tennessee during the growing seasons of 2013 and 2014. Corn, cotton, and soybean seed were treated with neonicotinoid insecticides. For each crop, plots of selected insecticide seed treatments were established in a randomized complete block design with four replicates. Row spacing varied from 0.76 to 1.02 m depending upon the crop and the location. Plots were four rows wide and 10–12 m in length. No additional neonicotinoids were applied to the crops during the sampling period. Leaf tissue was collected throughout the vegetative development of each crop until they reached reproductive growth stages (i.e., anthesis). The leaf tissue collected from each crop was the newest tissue on the plant, likely indicating at what concentrations neonicotinoid compounds were being translocated into new plant growth over time. The newest plant tissue was selected to measure the amount of insecticide moving into the plants between sample timings. If older tissue had been sampled, there would be a risk of measuring the accumulation of insecticides over time. Samples were analyzed to determine the concentration of neonicotinoid insecticides.

Corn experiments consisted of Dekalb[®] Genuity[®] 64–69 VT Triple Pro[®] (Monsanto Company, St. Louis, MO, USA) seed either not treated or treated with Poncho[®] 250, Poncho[®] 500, or Poncho[®] 1250 (clothianidin 0.25, 0.5, or 1.25 mg/seed, respectively, Bayer CropScience, Research Triangle Park, NC, USA). Samples were collected at growth stages V1 (first leaf), V3, V5, V7, V10, and VT (tassel). For each vegetative growth stage, the number represents how many leaf collars were visible on the corn stalk. At the VT growth stage, the corn plant reached maximum height and the last branch of the tassel emerged from the top of the plant [23]. For the V1 and V3 growth stages, sampling consisted of collecting 20 whole random plants within the plot. For the V5 and V7 growth stages, the uppermost fully expanded leaf was collected from 20 random plants within the plot. The uppermost fully expanded leaf was collected from 10 random plants for the V10 and VT growth stages. Corn plants at all locations reached the growth stages of V1, V3, and VT at approximately 5, 17, and 98 days after emergence, respectively.

For cotton experiments, Phytogen[®] 375 WRF (Dow AgroSciences, Indianapolis, IN, USA) was planted at all locations. Cotton treatments consisted of an untreated control, Avicta[®] Duo (thiamethoxam 0.34 mg/seed, Syngenta Crop Protection, Greensboro, NC, USA), and Aeris[®] (imidacloprid 0.375 mg/seed, Bayer CropScience, Research Triangle Park, NC, USA). Cotton leaves were sampled at the 1, 3, 5, 7, 9 true-leaf stages, and a final sample was collected at first flower (bloom). For the first and third true-leaf stage, the uppermost fully expanded leaf was taken from 20 random plants per plot. For all other growth stages, the uppermost fully expanded leaf was collected from 10 random plants per plot. For all locations, cotton vegetative growth stages ranged from 15 to 35 days after emergence. First bloom was approximately 65 days after emergence.

For the soybean experiments, Asgrow[®] 4606 (Monsanto Company, St. Louis, MO, USA) soybean seed were treated with Cruiser Maxx[®] Soybean (thiamethoxam 0.0778 mg/seed, Syngenta Crop Protection, Greensboro, NC, USA) and Poncho[®]/VOTiVO[®] (clothianidin 0.13 mg/seed, Bayer CropScience, Research Triangle Park, NC, USA). Untreated soybean seed was also planted to serve as a control. Soybean samples were collected at growth stages VC (cotyledon), V2, V4, V6, and R1 (early flowering) [24]. For the VC growth stage, 25 random whole plants were sampled from each plot. The uppermost fully expanded trifoliate was collected from 25 random plants per plot for the remaining growth stages. For all

locations, soybean vegetative growth stages ranged from 14 to 28 days after emergence. The R1 growth stage occurred at approximately 65 days after emergence. Additional planting information for corn, cotton, and soybean trials are available as Supporting Information.

All tissue samples were collected from the center two rows of each plot. Samples were placed in 3.8 L Ziploc® bags. New gloves were worn during the sampling of each plot to eliminate potential contamination. The sample bags were immediately placed in a cooler containing blue ice. Samples were transferred into a storage unit kept at $-10\text{ }^{\circ}\text{C}$ within 30 min of sampling and kept there until shipping for chemical analysis.

Soils samples were taken at the first and last sampling dates for each crop at each location. A soil probe (e.g., JMC 36" soil sampler, Clements Associates Inc., Newton, IA, USA) was driven 10 cm into the soil within the furrow where seeds were sown at 5 randomly selected spots within the center two rows of each plot. All 5 subsamples from each plot were placed in the same 3.8 L Ziploc® bag and thoroughly mixed. For each sample, three grams of the mixed soil were analyzed to determine concentrations of neonicotinoid insecticides.

All plant and soil samples were sent to the USDA AMS Science and Technology Laboratory Approval and Testing Division of the National Science Laboratory in Gastonia, NC to determine the levels of neonicotinoid insecticides in the plant tissue and soils. The plant tissues and soil samples were extracted for analysis of agrochemicals using a refined methodology for the determination of neonicotinoid pesticides using 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) [4,25–28]. Quantification was performed using external calibration standards prepared from certified standard reference material. Output was the concentrations of the neonicotinoid insecticides. The method detection limit was one part per billion (1 ng/g) for all parent compounds (Table A1). Non-detects were recorded as zero for calculating averages.

For plant tissue and for soil collected at each growth stage, analysis of variance was performed for neonicotinoid concentration for each treatment across all locations using PROC GLIMMIX of SAS (SAS Institute, version 9.3). Data were log transformed prior to analysis. The percent of positive detections was also determined for each treatment. Growth stage was considered a fixed effect in the model, and replication and location were designated as random effects. Degrees of freedom were calculated using the Kenward–Roger method. Means were calculated using the LSMEANS statement and separated based on Fisher's protected least significant difference ($\alpha = 0.05$).

3. Results

For corn, concentrations of neonicotinoid insecticides decreased sharply in leaf tissue as the plants grew. Concentrations of clothianidin in the Poncho® 250-treated plots varied among the different growth stages ($F = 27.0$; $df = 5, 50$; $p < 0.01$) (Table 1). Differences were also observed in the mean concentration of clothianidin detected at each growth stage for Poncho® 500- ($F = 25.5$; $df = 5, 50$; $p < 0.01$) and Poncho 1250®-treated plots ($F = 21.5$; $df = 5, 50$; $p < 0.01$) (Table 1).

Concentrations of clothianidin in corn leaf tissue from seed-treated corn dramatically decreased from the initial concentrations detected to what was detected at the VT growth stage. At the final sampling period, clothianidin was detected in 50 percent of the leaf tissue samples collected from the Poncho® 250-treated corn plants. These samples contained a mean concentration of clothianidin of $12 \pm 4.7\text{ ng/g}$ which was 99.3 percent less than the concentration of clothianidin initially detected at V1. Tissue from Poncho® 500-treated soybean seed contained $23 \pm 9.8\text{ ng/g}$ at the VT growth stage. There was a 99.3 percent decrease in the concentration of clothianidin that was initially detected at V1. Of the samples collected at VT from Poncho® 500-treated corn plants, 50 percent contained detectable levels of clothianidin. For leaf tissue from corn seed treated with Poncho® 1250, there was a 97.8 percent reduction in the concentration of clothianidin that was initially

detected at V1. All tissue sampled at the VT growth stage from Poncho[®] 1250-treated corn tested positive for clothianidin, and the mean concentration of clothianidin detected was 71 ± 22.3 ng/g.

Table 1. Mean concentration (ng/g) and percent detection of clothianidin detected in corn leaf tissue at each growth stage for each clothianidin seed treatment across all locations.

Growth Stage	0.25 mg Clothianidin/Seed		0.5 mg Clothianidin/Seed		1.25 mg Clothianidin/Seed	
	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection
V1	1625 (451) a	100%	3120 (1022) a	100%	3277 (762) a	100%
V3	650 (93) a	100%	1400 (174) a	100%	2758 (226) a	100%
V5	33 (7.8) bc	83%	90 (21.2) b	100%	307 (81) b	100%
V7	29 (3.3) b	100%	66 (7.15) b	100%	152 (41) bc	88%
V10	12 (3.0) cd	75%	22 (4.6) c	92%	63 (8.6) c	92%
VT	12 (4.7) d	50%	23 (9.8) c	50%	71 (22.3) c	100%

Means within a column followed by the same letter are not significantly different at ($p \leq 0.05$). Letters assigned based on log transformed statistics.

Cotton leaves from Aeris[®]-treated seed exhibited a substantial reduction in imidacloprid concentration as the plants grew larger ($F = 40.4$; $df = 5, 82$; $p < 0.01$) (Table 2). A similar reduction was also in the mean concentrations of thiamethoxam and clothianidin for plants treated with an Avicta[®] Duo seed treatment ($F = 33.2$; $df = 5, 58$; $p < 0.01$) (Table 2). It is important to note that clothianidin is a primary metabolite of thiamethoxam [29].

Table 2. Mean concentration (ng/g) and percent detection of neonicotinoids detected in cotton leaf tissue at each growth stage for the imidacloprid seed treatment (imidacloprid 0.375 mg/seed) and the thiamethoxam seed treatment (thiamethoxam 0.34 mg/seed) across all locations.

Growth Stage	0.375 mg Imidacloprid/Seed		0.34 mg Thiamethoxam/Seed			
	Imidacloprid		Thiamethoxam		Clothianidin	
	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection
1st Leaf	409 (71.2) a	100%	1640 (313) a	100%	250 (45.9) a	92%
3 Leaf	203 (33) a	100%	466 (61) a	100%	178 (11) a	100%
5 Leaf	63 (20.2) b	88%	117 (31) b	92%	27.4 (8.2) b	58%
7 Leaf	41 (16) b	75%	57 (14.4) bc	75%	20.2 (6.1) bc	63%
9 Leaf	7.5 (3.1) c	38%	22 (6.5) c	67%	5.6 (2.8) cd	50%
1st Flower	1.3 (0.5) c	38%	2.1 (1.1) d	33%	0.0 (0.0) d	0%

Means within a column followed by the same letter are not significantly different at ($p \leq 0.05$). Letters assigned based on log transformed statistics. Clothianidin mean concentrations reported for tissue from thiamethoxam treatment plots because clothianidin is a primary metabolite of thiamethoxam [29].

Concentrations of neonicotinoids in cotton leaf tissue dramatically decreased (fell to 2.1 ng/g or less) before the cotton plants began to flower. Leaf tissue collected from Aeris[®]-treated plots contained 1.32 ± 0.5 ng/g of imidacloprid at the final sampling stage, which was 99.7 percent less than the concentration of imidacloprid detected at first true leaf. At first flower, 38 percent of the samples from Aeris[®]-treated plants contained imidacloprid. When Avicta[®] Duo-treated cotton reached first flower, leaf tissue contained a mean concentration of thiamethoxam of 2.1 ± 1.1 ng/g. There was a 99.9 percent reduction in the concentration of thiamethoxam and 100 percent reduction in the concentration of

clothianidin that was initially detected at first true leaf. At the last sampled stage, 33 percent of the samples from Avicta[®] Duo-treated cotton tested positive for thiamethoxam.

Soybean leaves from CruiserMaxx[®]-treated seed also showed a large and rapid reduction in thiamethoxam and clothianidin concentrations as the plants grew ($F = 28.1$; $df = 4, 43$; $p < 0.01$ and $F = 29.0$; $df = 4, 43$; $p < 0.01$, respectively) (Table 3). Significant differences were also observed in the mean concentration of clothianidin detected at each growth stage for soybean grown from Poncho[®]/VOTiVO[®]-treated seed ($F = 21.3$; $df = 4, 42$; $p < 0.01$) (Table 3). Clothianidin was not detected in soybean leaf tissue treated for either neonicotinoid seed treatment by the time flowering occurred. Low levels of thiamethoxam persisted in tissue from the CruiserMaxx[®]-treated plants. Leaf tissue collected from CruiserMaxx[®]-treated soybean seed contained 2.2 ± 0.8 ng/g of thiamethoxam at the final sampling stage which was 99.9 percent less than the concentration of thiamethoxam detected at VC. One-half of CruiserMaxx[®]-treated soybean samples tested positive for thiamethoxam at the R1 growth stage.

Table 3. Mean concentrations (ng/g) and percent detection of neonicotinoids detected in soybean leaf tissue from plots containing the thiamethoxam seed treatment (thiamethoxam 0.0778 mg/seed) and the clothianidin seed treatment (clothianidin 0.13 mg/seed) at each sampled growth stage across all locations.

Growth Stage	0.0778 mg Thiamethoxam/Seed				0.13 mg Clothianidin/Seed	
	Thiamethoxam		Clothianidin		Clothianidin	
	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection
VC	3476 (847) a	100%	654 (167) a	100%	496 (177) a	100%
V2	273 (103) b	92%	22.3 (14.1) b	33%	14 (6.3) b	50%
V4	33 (30.2) c	50%	16.2 (6.6) b	50%	6.17 (3.0) bc	43%
V6	27.0 (18.0) c	63%	1.45 (1.0) bc	25%	0.7 (0.7) c	13%
R1	2.2 (0.9) c	50%	0.0 (0.0) c	0%	0.0 (0.0) c	0%

Means within a column followed by the same letter are not significantly different at ($p \leq 0.05$). Letters assigned based on log transformed statistics. Clothianidin mean concentrations reported for tissue from the thiamethoxam treatment plots because clothianidin is a primary metabolite of thiamethoxam [29].

Percent detections of the three neonicotinoids in soil from the three cropping systems varied greatly and were sporadic. Soil from untreated plots of all crops had some positive detections for all three neonicotinoids, creating background noise for the treatments (Tables 4–6). Imidacloprid was more frequently detected in untreated plots than clothianidin or thiamethoxam. Clothianidin was detected in soil from all treated corn plots at the V1 growth stage and was also detected in Poncho[®] 500- and Poncho[®] 1250-treated plots at the VT growth stage (Table 4). The corresponding neonicotinoid was detected in some soil samples from Aeris[®]- and Avicta[®] Duo-treated plots at the first true leaf stage and at similar frequencies at the first flower growth stage (Table 5). As in corn and cotton, the corresponding neonicotinoid was detected in most soil samples from CruiserMaxx[®] and Poncho[®]/VOTiVO[®] soybean treatments at both the VC and R1 growth stages (Table 6).

Neonicotinoids were not only detected in the soil of untreated plots, but they were also detected at low levels in the plant tissue of untreated corn (Table A2), cotton (Table A3), and soybean (Table A4) plots. Similarly, there were detections of neonicotinoid compounds in plant tissue that were different from the seed treatment in that plot. These detections may have been from plant uptake of residual neonicotinoids in the soil, or probably more likely reflect contamination during the treating, seed sorting, and the planting process.

Table 4. Mean concentrations (ng/g) and percent detection of neonicotinoids detected in soil from corn plots shortly after emergence (V1) and at early tasseling (VT) across all locations.

Treatment	Neonicotinoid Compound	Mean Concentration (SEM)		% Detection	
		V1	VT	V1	VT
Untreated	Clothianidin	5.5 (1.5)	0.0 (0.0)	75%	0%
	Imidacloprid	2.4 (1.6)	0.4 (0.4)	25%	13%
	Thiamethoxam	0.0 (0.0)	0.0 (0.0)	0%	0%
0.25 mg clothianidin/seed	Clothianidin	14 (3.0)	0.0 (0.0)	100%	0%
	Imidacloprid	1.1 (1.1)	0.5 (0.3)	13%	25%
	Thiamethoxam	0.0 (0.0)	0.0 (0.0)	0%	0%
0.5 mg clothianidin/seed	Clothianidin	37.2 (14.7)	18.6 (8.0)	100%	50%
	Imidacloprid	2.4 (2.4)	1.3 (1.1)	13%	25%
	Thiamethoxam	0.0 (0.0)	0.0 (0.0)	0%	0%
1.25 mg clothianidin/seed	Clothianidin	25.5 (7.7)	9.8 (4.9)	100%	38%
	Imidacloprid	2.7 (1.8)	0.5 (0.5)	25%	13%
	Thiamethoxam	0.0 (0.0)	0.0 (0.0)	0%	0%

Table 5. Mean concentrations (ng/g) and percent detection of neonicotinoids detected in soil from cotton plots at the first true-leaf stage and when plants began to flower across all locations.

Treatment	Neonicotinoid Compound	Mean Concentration (SEM)		% Detection	
		1st True Leaf	1st Flower	1st True Leaf	1st Flower
Untreated	Clothianidin	10.6 (6.5)	0.0 (0.0)	25%	0%
	Imidacloprid	4.1 (2.6)	0.3 (0.3)	42%	8%
	Thiamethoxam	3.1 (1.7)	0.0 (0.0)	25%	0%
0.375 mg imidacloprid/seed	Clothianidin	0.0 (0.0)	0.0 (0.0)	0%	0%
	Imidacloprid	25.1 (23)	95.5 (44)	75%	58%
	Thiamethoxam	0.0 (0.0)	0.0 (0.0)	0%	0%
0.34 mg thiamethoxam/seed	Clothianidin	0.0 (0.0)	3.1 (1.8)	25%	0%
	Imidacloprid	1.7 (0.7)	0.4 (0.2)	25%	42%
	Thiamethoxam	72.6 (43.8)	100 (43.2)	67%	58%

Table 6. Mean concentrations (ng/g) and percent detection of neonicotinoids detected in soil from soybean plots at the cotyledon stage (VC) and when plants began to flower (R1) across all locations.

Treatment	Neonicotinoid Compound	Mean Concentration (SEM)		% Detection	
		VC	R1	VC	R1
Untreated	Clothianidin	0.9 (0.9)	5.2 (4.5)	13%	17%
	Imidacloprid	1.0 (0.5)	1.2 (0.6)	38%	33%
	Thiamethoxam	0.0 (0.0)	0.0 (0.0)	0%	0%
0.0778 mg thiamethoxam/seed	Clothianidin	0.9 (0.9)	0.5 (0.5)	13%	8%
	Imidacloprid	0.0 (0.0)	0.4 (0.4)	0%	8%
	Thiamethoxam	16.4 (4.9)	16.4 (6.9)	100%	67%

Table 6. Cont.

Treatment	Neonicotinoid Compound	Mean Concentration (SEM)		% Detection	
		VC	R1	VC	R1
0.13 mg clothianidin/seed	Clothianidin	29.8 (7.5)	28.1 (4.6)	88%	100%
	Imidacloprid	1.3 (0.8)	1.3 (0.6)	38%	33%
	Thiamethoxam	0.0 (0.0)	6.6 (3.6)	0%	25%

4. Discussion

Neonicotinoid compounds are widely used as seed treatments on agronomic crops to provide control against many insect pests [1]. In the Midsouthern region of the United States, neonicotinoid seed treatments are commonly applied to corn, cotton, and soybean seed before planting [4,6,8,10]. These seed treatments are one of the few control options for early-season pests in these crops [4,6,8,10]. The systemic nature and persistence of these products continue to raise concerns about potential impacts on pollinators such as honey bees.

The goal of this research was to understand the change in neonicotinoid concentrations in plant tissue over time when neonicotinoid seed treatments were used in corn, cotton, and soybean plants and to compare these concentrations with published detections of concentrations in pollen and nectar from seed-treated crops. In all trials, neonicotinoid concentrations in leaf tissue had decreased dramatically by the time plants began flowering. Pollen and nectar data from previous studies show drastic reductions in neonicotinoid concentrations when compared to the concentrations derived from seed treatments in newly emerged crop tissue [4,17].

Newly emerged corn tissue from plots treated with the highest rate of the clothianidin seed treatment contained a mean clothianidin concentration of 3277 ng/g. At VT, only a mean of 71 ng/g of clothianidin was found in new leaf tissue, representing a 97.8 percent reduction in the initial concentration in the plant. The findings of 3.9 and 6.0 ng/g of clothianidin in pollen samples from Poncho[®] 250- and Poncho[®] 1250-treated corn [4] would indicate over a 99.7 percent reduction compared with what we detected at V1.

In the Midsouthern U. S., the lower labeled rates of Poncho[®] seed treatment (0.25 mg and 0.5 mg of ai/kernel) are more commonly applied to corn than the higher rate [30], and this would limit some exposure of pollinators to higher levels of clothianidin in pollen. Additionally, compared with many other crops, corn flowering occurs over a short period, lasting a maximum of two weeks within a field [31] during the R1 growth stage when pollen is shedding [23]. This short collection window for honey bees, the regional preference for lower rates of the Poncho[®] seed treatment, and the reduction in clothianidin concentrations within developing corn plants demonstrated in our research suggest that corn plants treated with Poncho[®] in the Midsouthern U.S. potentially pose a reduced risk of neonicotinoid exposure to foraging honey bees.

Leaf samples from cotton or soybean treated with neonicotinoid seed treatments also contained very low concentrations of neonicotinoids at the flowering stage compared with concentrations in recently emerged seedlings. Soybean nectar is considered to be of high quality and actively foraged on by honey bees [32], and Alburaki et al. [33] showed that honey bees also collect soybean pollen. For this reason, soybean fields are actively sought by commercial beekeepers in some areas of the Midsouth. The concentrations we detected in leaf tissues at flowering were low enough to suggest that, similar to the findings of Stewart et al. [4], little or no neonicotinoid residues would be found in cotton or soybean pollen and nectar. It appears neonicotinoid residues stemming from seed treatments are no longer present within soybean plants when they become attractive to foraging honey bees. This is also supported by the results of Camargo et al. [34]. These results do not rule out the possibility of higher concentrations of neonicotinoids being present in extra-floral nectar of cotton during the early growth stages of cotton. The results of the current experiment

contradict those of Jiang et al. [35] where a high percentage of samples contained low levels of thiamethoxam and imidacloprid following the use of those insecticides as seed treatments. During bloom, honey bees do not readily collect cotton pollen because of the size and shape of the pollen grain [33,36,37]. However, honey bees will collect nectar from cotton plants but have a strong preference for floral nectaries over extra-floral nectaries [38].

We found that neonicotinoid concentrations in the soil of plots having a seed treatment were sometimes higher than those in leaf tissues at the time of flowering. This was also observed by Stewart et al. [4]. This might result from a dilution of relatively low soil insecticide concentrations within rapidly growing plants. However, neonicotinoids present in soil during the reproductive stages of crops may not be available for uptake into the plant. Concentrations of clothianidin found in soil from seed-treated corn fields in the Midwestern United States were found to have limited plant bio-availability [21]. This was most likely due to time-dependent sorption, in which the pesticide residues became strongly bound to the soil matrix over time [21]. Soil-bound clothianidin concentrations were also found to not significantly increase or accumulate after four years of repetitive use of clothianidin seed treatments in corn production fields [21]. After 3 to 4 years of continued annual seed treatment use in corn fields, neonicotinoid residues plateau to mean concentrations of less than 6 ng/g [20]. These factors likely limit the exposure of foraging pollinators via neonicotinoid compounds in the soil of agricultural production.

These data help define the potential concentrations of neonicotinoid insecticides available in flowering crops stemming from insecticide seed treatments. They do not quantify exposure and toxicity, which are highly dependent on the attractiveness and willingness of honey bees to forage in these crops. It appears that in corn, cotton, and soybean seed treated with neonicotinoid insecticides, corn treated with high rates of clothianidin has the most potential to expose foraging honey bees to neonicotinoids through contaminated pollen. However, the concentrations available in pollen, as reported by others [4,17], do not appear sufficient to have measurable impacts on honey bees, especially if other floral resources were available. Similar conclusions have been made on similarly tested concentrations of imidacloprid in corn pollen [15]. Little to no measurable effects were found on honey bee colony health after colonies were fed pollen containing 5 ng/g of imidacloprid over a twelve-week period [15]. In another study, neonicotinoid-contaminated pollen resulted in mortality of worker bees that resulted in a temporary decline in population growth, but long term adverse effects were not detected. [39].

It appears that under the environmental conditions routinely encountered in the Midsouthern U.S. agroecosystems, the concentrations of neonicotinoids in corn, cotton, and soybean plants resulting from insecticide seed treatments are greatly reduced from early vegetative levels but impacts of concentrations measured in reproductive tissues were not examined against target or non-target arthropods. Our data are similar to those from Alford and Krupke [40] suggesting that the value of neonicotinoid seed treatments in controlling pests is short lived. However, the benefits of treatment to the crop often persist longer because of more vigorous seedling growth in the absence of pests that were controlled by the neonicotinoid seed treatment [6,8,10]. Thus, infestation of even susceptible pests that occur beyond the first few weeks may not be effectively controlled.

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Appendix A

Corn experiments were planted at the Mississippi State University R. R. Foil Plant Science Research Center in Starkville, MS, the Mississippi State University Delta Research and Extension Center in Stoneville, MS, USA and the University of Tennessee West Tennessee Research and Education Center in Jackson, TN, USA in the 2013 growing season. Upland cotton was planted at the University of Arkansas Lon Mann Cotton Research Station in Marianna, AR in 2013, the Mississippi State University R. R. Foil Plant Science Research Center in Starkville, MS, USA and the Mississippi State University Delta Research and Extension Center in Stoneville, MS, USA in 2014. In 2014, cotton treated with imidacloprid at a rate of 0.375 mg/seed was sampled at the University of Tennessee Research and Education Center in Milan, TN, USA. Soybean was planted in 2013 at the Mississippi State University R. R. Foil Plant Science Research Center in Starkville, MS, USA, the Mississippi State University Delta Research and Extension Center in Stoneville, MS, USA and the University of Arkansas Lon Mann Cotton Research Station in Marianna, AR, USA. Cotton tests were performed on a lighter textured silt loam soil. Corn and soybean trials were performed on a range of soil types ranging from silt loam to Sharkey clay.

Table A1. The pesticide residues and analytical levels of detection (LOD) that were screened for during analyses.

Pesticide Residue	LOD (ng/g)
Acetamiprid	2.0
Clothianidin	1.0
Dinotefuran	2.0
Flonicamid	8.0
Imidacloprid	1.0
Thiacloprid	1.0
Thiamethoxam	1.0

Table A2. Mean concentration (ng/g) and percent detection detected in corn leaf tissue from untreated plots at each sampled growth stage across all locations.

Growth Stage	Clothianidin		Imidacloprid		Thiamethoxam	
	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection
V1	64.6 (19.2)	100%	1.6 (0.8)	33%	60.5 (16.6)	100%
V3	11.6 (4.2)	75%	1.8 (1.1)	50%	13.5 (2.1)	100%
V5	1.1 (1.1)	8%	0.9 (0.5)	25%	9.5 (3.6)	100%

Table A2. Cont.

Growth Stage	Clothianidin		Imidacloprid		Thiamethoxam	
	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection
V7	2.7 (1.3)	50%	0.0 (0.0)	0%	0.0 (0.0)	0%
V10	0.6 (0.4)	17%	0.0 (0.0)	0%	0.4 (0.3)	17%
VT	0.0 (0.0)	0%	0.0 (0.0)	0%	0.0 (0.0)	0%

Table A3. Mean concentration (ng/g) and percent detection detected in cotton leaf tissue from untreated plots at each sampled growth stage across all locations.

Growth Stage	Clothianidin		Imidacloprid		Thiamethoxam	
	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection
1st Leaf	1.9 (1.9)	8%	4.3 (2.7)	42%	2.8 (1.6)	33%
3 Leaf	0.0 (0.0)	0%	4.4 (1.4)	75%	0.8 (0.8)	13%
5 Leaf	1.7 (0.9)	25%	1.7 (0.9)	33%	1.7 (1.2)	17%
7 Leaf	3.8 (3.8)	13%	0.8 (0.6)	25%	0.0 (0.0)	0%
9 Leaf	0.0 (0.0)	0%	0.0 (0.0)	0%	0.0 (0.0)	0%
1st Flower	0.0 (0.0)	0%	0.2 (0.2)	8%	0.0 (0.0)	0%

Table A4. Mean concentration (ng/g) and percent detection detected in soybean leaf tissue from untreated plots at each sampled growth stage across all locations.

Growth Stage	Clothianidin		Imidacloprid		Thiamethoxam	
	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection	Mean Concentration (SEM)	% Detection
VC	0.0 (0.0)	0%	1.2 (0.2)	88%	0.0 (0.0)	0%
V2	0.0 (0.0)	0%	0.1 (0.1)	8%	1.0 (0.6)	33%
V4	0.0 (0.0)	0%	0.3 (0.3)	13%	0.0 (0.0)	0%
V6	0.0 (0.0)	0%	0.7 (0.5)	25%	3.8 (2.9)	25%
R1	0.0 (0.0)	0%	0.1 (0.1)	8%	0.4 (0.4)	8%

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