

## ARTHROPOD MANAGEMENT AND APPLIED ECOLOGY

### Insecticide and Fungicide Residues Following Foliar Application to Cotton and Soybean

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#### ABSTRACT

**The control of target pests and impact on non-target arthropods, including pollinators, is affected by the persistence of pesticides on plants following an application. A study was conducted in Tennessee to investigate the levels of pesticide residues on cotton, *Gossypium hirsutum* L., and soybean, *Glycine max* (L.), following a foliar application made during early flowering. Residues of four classes of insecticides and three classes of fungicides were assessed at 1, 24, 72, 144, and 216 h after application on cotton leaves, anthers of cotton flowers, and soybean flowers. Active ingredients included acephate, imidacloprid, lambda-cyhalothrin, chlorantraniliprole, fluxapyroxad, pyraclostrobin, and propiconazole. Initial pesticide residues on cotton leaves were many times greater than those on cotton anthers or soybean flowers. With the exception of chlorantraniliprole on cotton leaves, fungicide residues persisted longer than insecticides. Also, pesticide residues on soybean flowers degraded more slowly than those on cotton leaves or anthers. For cotton leaves, insecticide residues decreased sharply within 24 h after application except for chlorantraniliprole. All pesticide residues on cotton anthers were dramatically lower 24 h after application, indicating little systemic movement to pollen. By 216 h after application, and considerably sooner in most scenarios, pesticide residues on cotton and soybean had diminished by 90% or more. The implications of these results on pest management and pollinator safety are discussed.**

**F**oliar-applied pesticide applications to control arthropod pests and fungal plant pathogens

are made frequently during the flowering stage of many crops. Cotton, *Gossypium hirsutum* L., and soybean, *Glycine max* (L.), are self-pollinating, but the flowers are attractive to an array of bees including honey bees, *Apis mellifera* L. (Hymenoptera: Apidae) (Gill and O’Neal, 2015; Parys et al., 2020). Cotton and soybean also are used by beekeepers for honey production (USDA ARS, 2017). Mass flowering crops are known to enhance pollinator densities at a landscape level (Holzschuh et al., 2013; Westphal et al., 2003), and because cotton and soybean are grown on a wide geographical basis, it follows that negative effects of pesticide applications might have the opposite effect on pollinator densities. Insecticide applications also can have unintended impacts on beneficial arthropods, resulting in pest outbreaks (Hill et al., 2017; Pisa et al., 2014). Neonicotinoids have been under extreme scrutiny by being linked to declines in pollinator health (Cressey, 2017; Pollinator Network @ Cornell, 2020). However, there are concerns about other classes of pesticides, including fungicides and their potential for synergism with insecticides causing increased toxicity to non-target organisms (Blacquière et al., 2012; Thompson et al., 2014; Tosi and Nieh 2019).

Pesticide residues on crops are thoroughly investigated during the registration process. These data focus on food and environmental safety, including assessments of potential impact on non-target species including pollinators. A substantial amount of information is known about pesticide residues when applied under field or greenhouse conditions. For example, Djouaka et al. (2018) reported more than a 95% reduction in lambda-cyhalothrin residues on lettuce (*Lactuca* sp.) after 7 d and no detection by 9 d after application. Barik et al. (2010) reported half-life values of approximately 5.5 and 4.8 d for thiamethoxam and lambda-cyhalothrin, respectively, following foliar application in rice (*Oryza sativa* L.). Szpyrka et al. (2017) reported a 16- to 17-d half-life of the diamide insecticide chlorantraniliprole in apples, *Malus domestica* Borkh. Chlorantraniliprole was shown

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to cause substantial mortality in assays with corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), on soybean foliage beyond 30 d after application (Adams et al., 2016). Approximately 50% of the fungicide fluxapyroxad was present 7 d after application on perilla, *Perilla frutescens* var. *japonica* Hara, grown in a greenhouse (Noh et al., 2019). Butler et al. (2018) showed residues of azoxystrobin, a commonly used QoI (strobilurin) fungicide, decreased exponentially on soybean leaves following a foliar application with a predicted half-life of 2.54 to 4.82 d, depending upon test conditions.

The objective of this study was to gain a better understanding about the persistence of commonly used insecticides and fungicides in cotton and soybean when applied under field conditions. This has obvious implications on the potential of applications to control pests and harm populations of beneficial arthropods, including pollinators. It might also provide insight to approaches that mitigate the negative effects of pesticide applications on pollinators.

## MATERIALS AND METHODS

This study was performed at the West Tennessee Research and Education Center in Jackson, TN to evaluate the persistence of pesticide residues on cotton and soybean after a foliar application. The cotton and soybean varieties used in this study were PHY 425 RF (Phytogen, Indianapolis, IN) and P48A60X (Pioneer, Johnston, IA), respectively. Two replicates of cotton and soybean, each eight rows wide and approximately 30 to 35 m long, were planted in alternating strips on 13

June 2019. The row spacing was 96.5 cm. Plots were managed based on standard production practices but making certain, with the exception of seed treatments, that the active ingredients being evaluated were not applied before or during the experiment.

A tank mixture of three insecticide and two fungicide products was applied during flowering. The application was made mid-morning to the center four rows of each plot using a research-grade plot sprayer at an application volume of 87.7 l/ha, pressure of 276 kpa, and using 8001 flat fan nozzles, and a ground speed of 5.63 km/h. Boom height was set at approximately 30 cm above the canopy, and applications were made when winds were nearly imperceptible (< 3 km/h). Nozzles were spaced at 48.3 cm, and at the pressure indicated above, are designed to deliver a fine droplet with a volume median diameter of approximately 200 microns. In soybean, this application was made on 23 July at the R2 growth stage (full bloom). The application was made in cotton on 30 July, approximately 7 d after blooms first appeared on most plants. The pesticide products used, active ingredients, and use rates are shown in Table 1. These products were applied at rates typical or above the normal field-use rates. Products were selected to represent multiple classes of insecticides and fungicides that are used commonly in cotton and soybean (Table 1). With the exception of the insecticide lambda-cyhalothrin, all pesticides used in this study are known to have some plant-systemic or translaminar properties. Rainfall and temperature data were recorded during the study from a weather station located approximately 1 km from the study site.

**Table 1.** Trade name, active ingredient (PPB limit of detection), experimental rate, and pesticide class of materials applied to flowering cotton and soybean to evaluate the persistence of residues

Trade Name (Manufacturer) <sup>Z</sup>	Active Ingredients (LOD)	Rate (g ai/ha)	Pesticide Class (IRAC or FRAC Classification) <sup>Y</sup>
Orthene 97 SP (Amvac)	Acephate (50)	1,087	Organophosphate (1b)
Admire Pro (Bayer CropScience)	Imidacloprid (5)	68	Neonicotinoid (4A)
Besiege (Syngenta)	Chlorantraniliprole (14)	73	Diamide (28)
	Lambda-cyhalothrin (24)	37	Synthetic pyrethroid (3A)
Priaxor Xemium (BASF)	Fluxapyroxad (2)	49	Pyrazole carboxamide (7)
	Pyraclostrobin (2)	97	QoI, strobilurin (11)
Tilt (Syngenta)	Propiconazole (5)	126	Demethylation inhibitor (3)

<sup>Z</sup> Amvac (Newport Beach, CA), Bayer CropScience (St. Louis, MO), Syngenta (Greensboro, NC), BASF (Florham Park, NJ).

<sup>Y</sup> Insecticide (IRAC) or Fungicide (FRAC) Resistance Action Committee.

Plant tissues for pesticide analysis were collected from multiple plants within the center two rows of each plot and included 20 cotton leaves from the third node below the terminal, anthers from at least 25 white cotton flowers, and 75 whole soybean flowers. Samples were collected immediately prior to application and 1, 24, 72, 144, and 216 h after application. However, only data collected prior to application and 1, 72, 144, and 216 h after application are presented for soybean due to a processing error. Samples were processed and weighed immediately after collection and stored in a -80 °C freezer. The samples were subsequently shipped to the U.S. Department of Agriculture, Agricultural Marketing Service National Science Laboratory (Gastonia, NC) where the plant tissues were finely ground and processed for liquid chromatography coupled with tandem mass spectrometry to quantify residual pesticide concentrations (USDAAMS, 2020; Zhang et al., 2011). The laboratory-reported limits of detection for each active ingredient are shown in Table 1. Trace detections indicating the presence of an active ingredient at an unquantifiable level below the limit of detection were also reported. When calculating average residue levels, we assumed a concentration of zero for reports of no detection, and for trace detections, we assumed a concentration equal to one-half the limit of detection.

## RESULTS

Minimum daily temperatures ranged from 15.0 to 20.6 °C, and high daily temperatures ranged from 26.7 to 32.8 °C during this study. One rainfall event (22.6 mm) was recorded on 29 July, 6 d after the pesticide application to soybean and prior to the application to cotton. A trace rainfall event (< 1 mm) occurred on 2 August, after all soybean samples were collected, and therefore could have only affected the results for cotton.

For samples collected prior to application, no residues were detected on cotton anthers or on soybean blooms. In the two pre-application samples of cotton leaves, acephate was present at a concentration of 601 and 739 ppb, and chlorantraniliprole was detected in one sample at 14 ppb. All other pesticides were either not detected or detected at trace levels (Table 2). Pre-application detections were likely the result

of contamination from previous applications because the spraying equipment is routinely used for other applications. Pre-application pesticide residues occurred at trivial levels relative to the initial residues found after application (Table 3), and thus, they were ignored.

As expected, pesticide residues were highest in samples collected 1 h after application (Tables 2 and 3). Residues were highest on cotton leaves. Depending on the active ingredient, initial residues on cotton leaves were 36 to 121 times higher than those on cotton anthers and 16 to 55 times higher than found on soybean flowers.

Figure 1 shows pesticide residues over time as a percentage of the maximum levels observed at 1 h after application. Chlorantraniliprole residues on cotton leaves decreased relatively linearly during the study (Fig. 1). In contrast, the residue levels of other insecticides decreased by more than 80% by 24 h after application and continued to decline thereafter. Fungicide residues generally decreased more slowly than insecticides, except chlorantraniliprole, but only persisted at low levels by 144 h (6 d) after application (Fig. 1). For all pesticides, residues levels on cotton leaves decreased by more than 95% by 216 h (9 d) after application. Keeping in mind that initial pesticide residues on cotton anthers were much lower than those on leaves (Table 3), the reduction of residues on cotton anthers was dramatic, ranging from 88 to 98% for all pesticides by 24 h after application (Fig. 1). In contrast, pesticide residues on soybean flowers, initially much lower than those on cotton leaves but greater than those on cotton anthers, decreased more slowly and relatively linearly (Fig. 1).

Although there were only two replicates, there was generally good agreement of the residue values. For example, at 1 h after application on cotton leaves, replicate values for each active ingredient varied by an average of 9.9% from the mean, with a range of 1.6 to 13.2% for individual ingredients. There was also good fidelity between replicates in the reduction of pesticide concentrations over time. For example, acephate concentrations for the two replicates of cotton leaves at 24 h after application were 9.4 and 11.4% of the average concentration at 1 h. Similarly, imidacloprid concentrations for the two replicates of cotton anthers collected at 24 h application were 0.0 and 6.7% of the average concentration at 1 h.

**Table 2.** Pesticide residues (ppb) detected in all cotton and soybean samples collected during 2019 immediately before (Pre) and at different times (h) after a foliar application; T, indicates unquantifiable trace amounts below the lower limit of detection

Date	Timing	Sample	Acephate	Chlorantran.	L-cyhalothrin	Fluxapyroxad	Imidacloprid	Propiconazole	Pyraclostrobin
7/30/19	Pre	Cot Leaves 1	601	14	T	T	T	0	T
	Pre	Cot Leaves 2	739	T	T	T	T	0	T
	Pre	Cot Anthers 1	0	0	0	0	0	0	0
	Pre	Cot Anthers 2	0	0	0	0	0	0	0
7/30/19	1 h	Cot Leaves 1	316,000	33,600	12,700	16,700	15,500	37,500	28,200
	1 h	Cot Leaves 2	243,000	27,600	12,300	12,300	12,300	29,200	20,900
	1 h	Cot Anthers 1	9,870	477	101	269	315	511	526
	1 h	Cot Anthers 2	5,730	447	104	204	219	392	397
7/31/19	24 h	Cot Leaves 1	35,800	21,700	2,290	6,510	693	6,680	10,800
	24 h	Cot Leaves 2	41,600	23,900	2,690	7,100	892	8,550	12,300
	24 h	Cot Anthers 1	0	T	T	0	0	24	9
	24 h	Cot Anthers 2	957	T	T	8	18	35	6
8/2/19	72 h	Cot Leaves 1	33,300	18,800	797	5,030	131	1,150	4,720
	72 h	Cot Leaves 2	26,400	19,000	800	4,460	164	1,180	4,630
	72 h	Cot Anthers 1	0	24	0	5	13	0	13
	72 h	Cot Anthers 2	0	31	0	6	18	0	8
8/5/19	144 h	Cot Leaves 1	7,960	6,630	238	969	47	890	1,430
	144 h	Cot Leaves 2	10,800	9,920	265	1,030	51	751	1,310
	144 h	Cot Anthers 1	0	T	0	7	0	11	4
	144 h	Cot Anthers 2	0	T	0	0	8	0	3
8/8/19	216 h	Cot Leaves 1	2,820	1,760	108	594	10	588	868
	216 h	Cot Leaves 2	585	344	32	207	18	203	316
	216 h	Cot Anthers 1	0	26	0	0	0	0	0
	216 h	Cot Anthers 2	50	13	25	2	5	5	3
7/23/19	Pre	Soy Flowers 1	0	0	0	0	0	0	0
	Pre	Soy Flowers 2	0	0	0	0	0	0	0
7/23/19	1 h	Soy Flowers 1	15,800	1,430	534	222	345	1,440	421
	1 h	Soy Flowers 2	18,300	1,680	612	304	450	789	576
7/26/19	72 h	Soy Flowers 1	5,880	277	187	110	200	676	246
	72 h	Soy Flowers 2	8,400	464	242	154	319	1,170	296
7/29/19	144 h	Soy Flowers 1	2,290	82	T	45	47	63	76
	144 h	Soy Flowers 2	3,330	217	43	125	100	170	190
7/29/19	144 h	Soy Leaves 1	9,410	2,670	273	613	19	707	950
	144 h	Soy Leaves 2	12,800	5,380	489	864	34	1,164	1,620
8/1/19	216 h	Soy Flowers 1	903	62	T	14	14	14	23
	216 h	Soy Flowers 2	1,290	75	T	19	31	27	37
8/1/19	216 h	Soy Leaves 1	3,580	518	93	250	8	85	311
	216 h	Soy Leaves 2	8,560	3,410	289	648	35	416	765

**Table 3.** Average pesticide residues (ppb) detected on different plant tissues at 1 h after application; individual sample values are shown in Table 2

Active ingredient	Cotton leaves	Cotton anthers	Soybean flowers
Acephate	279,500	7,800	17,050
Imidacloprid	13,900	267	398
Chlorantraniliprole	30,600	462	1,555
Lambda-cyhalothrin	12,500	103	573
Fluxapyroxad	14,500	237	263
Pyraclostrobin	24,550	461	499
Propiconazole	33,350	452	1,115

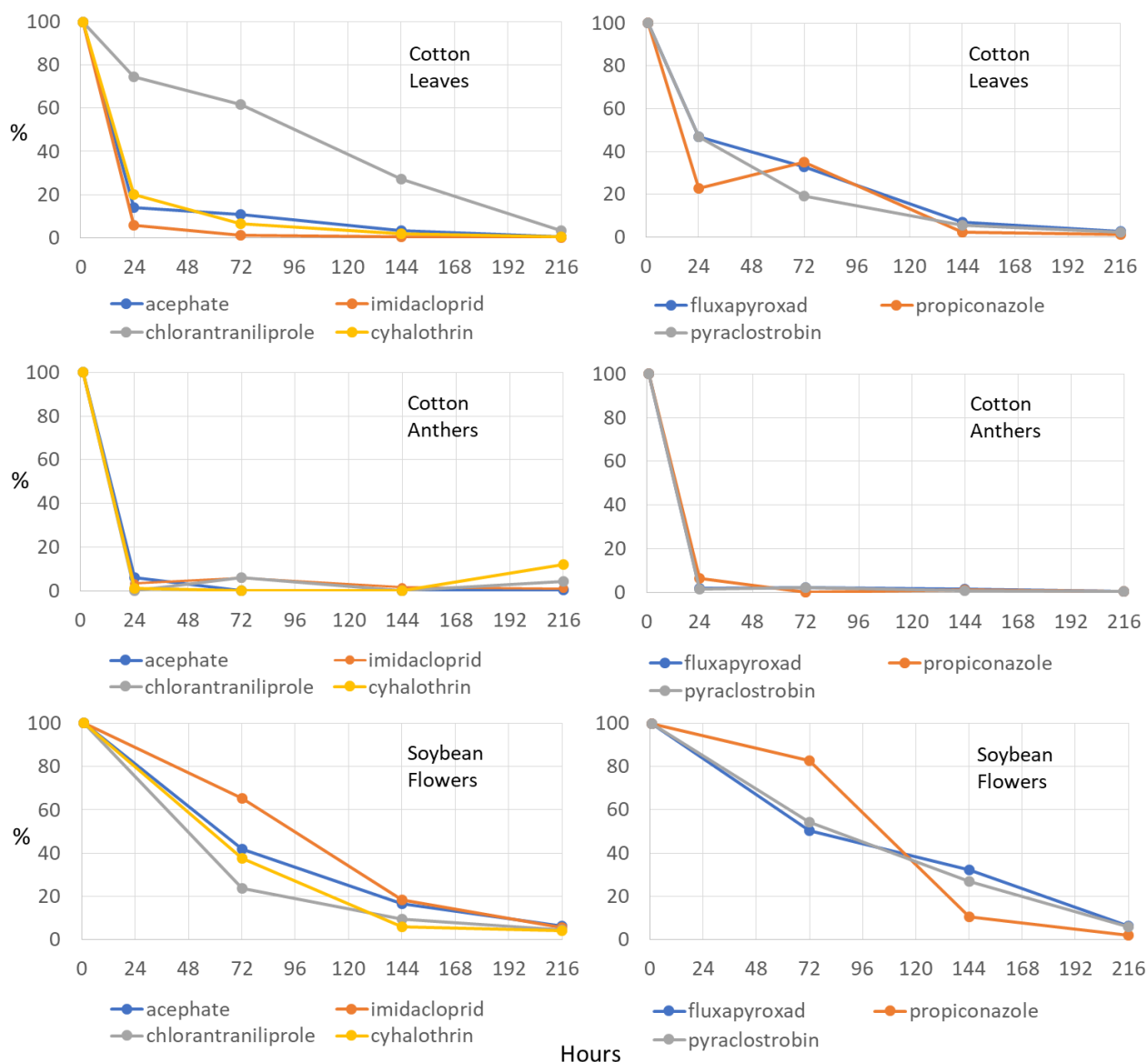


Figure 1. Mean percent insecticide (left) and fungicide (right) residues on cotton leaves, cotton anthers, and soybean flowers at different times (hours) after a foliar application during early flowering. Results are relative to pesticide residues detected 1 h after application (Table 3).

## DISCUSSION

Our results indicated a rapid reduction in the residual concentration of multiple insecticides and fungicides in cotton following a foliar application, especially for insecticides and residues found in anthers. Pesticide residues on soybean flowers degraded more slowly. Although there were only two replicates, there was good consistency in the initial pesticide concentrations found between the replicates, varying by 1.6 to 13.2% of the mean value depending upon the active ingredient (Table 2). Because pesticide residues declined quickly for some active ingredients,

especially in cotton, the subsequent variation between replicates was small in relation to initial pesticide concentrations. Weather probably did not substantially impact our results. Only a trace amount of rain (< 1 mm) was recorded at 3 d after treatment during the study with cotton. There was a substantial rainfall at 6 d after treatment in soybean, but this occurred after samples had been collected on that day. Thus, only data for soybean flowers collected at 216 h after application would have been affected.

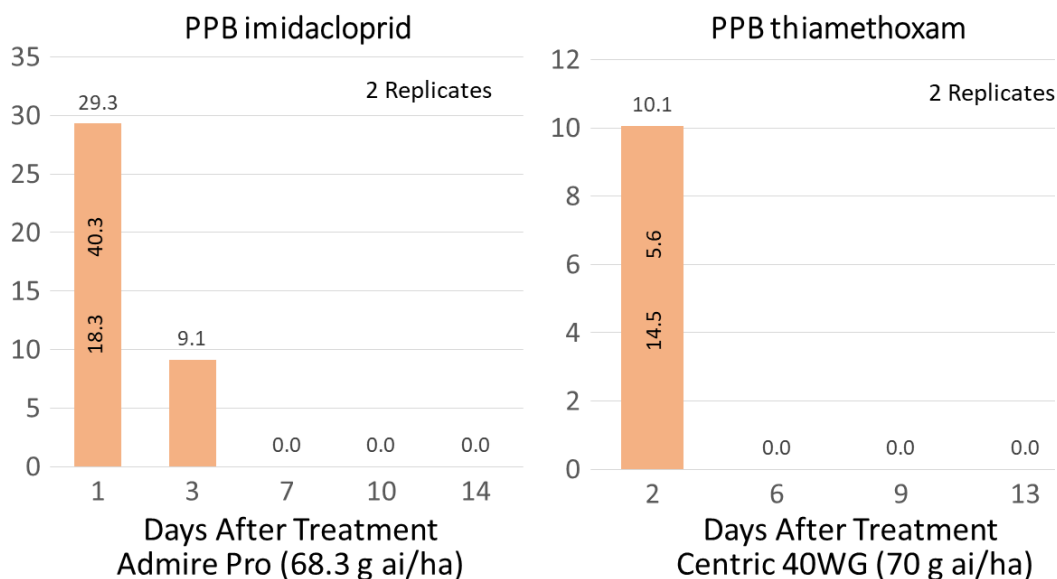
We did not test for the presence of metabolites, and insecticide metabolites could have insecticidal activity including some imidacloprid metabolites and

methamidophos, a metabolite of acephate (Extoxnet, 2020; Nauen et al., 2001). Thus, the levels of residues we detected could underestimate potential exposure of target pests or pollinators to pesticide residues. However, because the assay methods involved grinding of plant tissues and the use of solvents for extraction, they could overestimate the bioavailability, and thus exposure, of pesticide residues encountered by arthropods. The metabolites of imidacloprid and thiamethoxam were not detected in cotton anthers in a similar but unpublished study (Fig. 2). In other studies with neonicotinoids, metabolites were detected at much lower levels than the parent compound (Stewart et al., 2014; Vineyard and Stewart, 2017). Similarly, Bull (1979) found that methamidophos and other metabolites represented a fraction of the residues found following the application of acephate to cotton. Interestingly, he also found that < 6% of the original acephate dose was recovered 48 h after application by rinsing the surface of leaves, with the rapid drop primarily attributed to absorption into the plant.

At 1 h after the foliar application, we detected much higher concentrations of pesticides on cotton leaves compared with cotton anthers or soybean flowers. This is not surprising considering these leaves were collected from the upper canopy and presumably intercepted many spray droplets. This almost certainly occurred in soybean as well. Indeed, two samples of soybean leaves collected 144 and 216 h after application were inadvertently submitted for analysis. Pesticide concentrations on these

leaves were substantially higher than those on flowers, except for imidacloprid (Table 2). Interestingly and unexplained is why the ratio of initial pesticide residues collected 1 h after application in different parts of the canopy varied by active ingredient. For example, the concentration of imidacloprid residues on cotton leaves was approximately 52-fold higher than on anthers, whereas the concentration of lambda-cyhalothrin was 121-fold higher. This could be related to changes in the sensitivity of residue analyses resulting from differences in sample mass or the type of tissue assay. The fresh weight biomass of cotton anthers and soybean flowers samples ( $\approx 1$  g) were below optimum levels suggested by the laboratory.

In general, insecticide residues degraded more quickly than fungicide residues. However, insecticide residues on soybean flowers generally degraded more slowly than those in cotton. This could be because soybean flowers are protected from sunlight by the canopy leaves, thus reducing photodegradation, a process well known to degrade pesticides (Katagi, 2004). Residues found on cotton anthers decreased quickly (Fig. 1). This was expected and consistent with the previously mentioned unpublished study (Table 2, Fig. 2). Anthers should be directly exposed to pesticides only on the day of application because a cotton flower opens in the morning and only remains open for one day (Williams and Bange, 2018). Indeed, in the authors' experience, cotton flowers close by nightfall, remain closed, and are not attractive to pollinators the following day. We chose cotton anthers not



**Figure 2.** Concentration (ppb) of imidacloprid and thiamethoxam in cotton anthers that were found after a foliar application was made to cotton during early bloom. No metabolites of imidacloprid or thiamethoxam were detected in a full screening of neonicotinoid compounds (unpublished data).

only because pollen is a resource for pollinators but also because residues found in a new, white flower's anthers after the day of application would indicate systemic movement. Our data suggest there is little systemic movement of pesticide residues into cotton pollen or that systemic activity is diluted within plant biomass that, during flowering, is many thousands of times greater than the amount of active ingredient applied. That some residues, albeit at low levels, were found on anthers throughout the study might indicate physical movement, perhaps by dew or the trace amount of rainfall that occurred, especially because this also was observed for lambda-cyhalothrin, a non-systemic insecticide.

Our results document a rapid decrease in pesticide residues and particularly insecticides within 24 h of application. For insecticides, the exception was chlorantraniliprole, which persisted at noticeably higher levels on cotton leaves up to 144 h after application. This was not unexpected given the well-documented residual control provided by this diamide against many lepidopteran pests compared with alternative insecticides (Adams et al., 2016; Steckel and Stewart, 2016). Interestingly, the slower degradation of chlorantraniliprole was not observed on soybean flowers. It should be noted that, because all leaves were collected from the third node down from the cotton terminal, the leaves collected at 144 and 216 h would have been relatively small, less developed leaves located higher in the plant's terminal at the time of application. Thus, some of the reduction in residue levels likely resulted from an increasing leaf size after application. This applies to flower buds in cotton (squares) or developing soybean buds that would have been subsequently collected as flowers.

Systemic or translaminar movement of the pesticides applied in this study did not result in high residues within cotton anthers, at least beyond the day of application. This has obvious implications on pollinator safety, especially if this also reflects a lack of systemic movement into nectar. Dively and Kamel (2012) reported considerably higher residues resulting from systemic movement of neonicotinoids into pollen compared with nectar in pumpkin (*Cucurbita pepo* L.), but this relationship might not hold true for other crops or other pesticides. Regardless, recommendations to make insecticide applications later in the day when pollinators are less active or when floral resources are less available appear to have merit. Unlike bees, which are primarily day active, this could be less beneficial for Lepidoptera and other pollinating insects that are

active during the night (Macgregor and Scot-Brown, 2020; Manning and Cutler, 2013). Regardless, delaying pesticide application until later in the day could provide more protection of pollinators than expected given the rapid reduction of residues, particularly in cotton, within 24 h. This would seem especially valuable in crops where blooms close as the day progresses or those, like cotton, where individual flowers are open for only 1 d. However, pesticides applied later in the day could initially degrade more slowly because they would have less exposure to daylight. Presumably, control of arthropod pests would be less impacted by applications made later in the day unless they are strictly associated with flowers and floral resources.

The Environmental Protection Agency (EPA, 2012) previously defined levels of concern for imidacloprid, thiamethoxam, and clothianidin for concentrations in pollen or nectar that, if exceeded, pose a risk of causing acute mortality to adult honey bees. These levels are approximately 170 ppb in pollen and 5 ppb in nectar and are partly based on pollen and nectar consumption rates by worker bees (Stewart et al., 2014). Using imidacloprid, the honey bee, and the residues we observed in cotton as a model, the residues found on cotton anthers were well below levels of concern for neonicotinoid concentration in pollen except on the day of application. Thus, expected acute mortality of honey bees foraging on contaminated pollen would be low the day after application. Residues near the level of concern for nectar ( $\approx 5$  ppb) were present for several days, assuming concentrations in nectar were similar to those in anthers. However, feeding studies consistently show that short duration exposure of honey bee colonies to these low residues of imidacloprid in sugar water have little or no measurable effect on colony health (Meikle et al., 2016).

Contact exposure to insecticide residues on treated plant surfaces is expected to cause substantial acute mortality to many target and non-target arthropods on the day of application. Imidacloprid residues observed on cotton leaves in our study were  $> 17$ -fold higher at 1 h after application ( $\approx 14,000$  ppb) than they were 24 h after application ( $\approx 790$  ppb). Because honey bees are approximately 5 to 20 times less sensitive to nitro-containing neonicotinoid when exposed topically (Blacquièrre et al., 2012; Ecotox, 2020), the imidacloprid residues we detected on cotton leaves at 72 h after application ( $\approx 150$  ppb) would not be expected to cause significant acute mortality. Indeed they were below levels of concern even for bees consuming contaminated pollen.

An arthropod might be exposed by direct topical exposure to spray droplets, through contact with treated plant materials, and/or by ingestion of treated plant tissues. Thus, caution should be used when trying to predict mortality based on residue levels found on plants, especially without knowing how and how long an arthropod might interact with various plant structures. However, our data suggest that mortality caused by many insecticides is primarily the result of exposure during the few days after application. It also supports a conclusion that many foliar pesticide applications that are made to fields with substantial plant biomass are primarily affecting pests by contact activity or by localized, translaminar activity rather than systemic activity, even if these pesticides have systemic properties.

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