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Research

Determining *Frankliniella fusca* (Thysanoptera: Thripidae) Egg Distribution in Neonicotinoid Seed-Treated Cotton

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Abstract

Frankliniella fusca (Hinds) (Thysanoptera: Thripidae) is an early-season cotton pest. Seedlings are injured by larvae, which hatch from eggs oviposited into seedlings and feed on developing plant tissue. Better understanding *F. fusca* oviposition in cotton may improve their management and address new challenges such as resistance to neonicotinoid seed treatments (NSTs). Cotton seedlings exposed to *F. fusca* were either cleared and stained to determine egg density and location, or dissected and washed to determine larval distribution. Experiments were conducted in the greenhouse with a susceptible population and field with a NST-resistant population. Eggs of both populations were recovered predominantly in cotyledons. Larvae were more uniformly distributed on seedlings. On NST seedlings, oviposition by the susceptible population was reduced and preference shifted to true leaves. NSTs did not alter egg placement by the resistant population. These findings suggest that injury to cotton seedlings is primarily caused by *F. fusca* emerging on the cotyledons, and then moving to developing leaves. The oviposition shift in NST plants correlates with how systemic NSTs have been reported to concentrate in cotyledons. This can better inform management tactics in cotton, such as well-timed foliar sprays, which, given the current resistance issue, are needed to maintain effective thrips management.

Key words: imidacloprid, thiamethoxam, resistance management, tobacco thrip, *Gossypium hirsutum*

Neonicotinoid-resistant tobacco thrips, *Frankliniella fusca* (Hinds), (Thysanoptera: Thripidae) present an increasingly serious obstacle to affordable and sustainable cotton production in the southeastern United States (Huseth et al. 2016). *Frankliniella fusca* is an early-season pest of cotton, with adult insects ovipositing on young cotton seedlings, and plant injury resulting from larval feeding (Cook et al. 2011). This injury can deform plants, disrupt apical dominance, and in serious cases, kill seedlings (Gaines 1934). Currently, control of these insects is primarily accomplished through neonicotinoid seed treatments (NSTs), with active ingredients such as thiamethoxam and imidacloprid (Elbert et al. 2008, Huseth et al. 2016). Although NSTs effectively controlled these insects for nearly 20 yr, their efficacy has eroded due to widespread resistance to both active ingredients (Huseth et al. 2016). This has caused growers to rely on follow-up foliar sprays of organophosphates such as acephate (Reisig 2018), greatly increasing insecticide input into these systems (Huseth et al. 2016).

Closely examining aspects of *F. fusca* biology in cotton may offer insight into both how NST resistance developed in the field and what measures can be used to more sustainably combat this insect in the future. One area of particular interest is *F. fusca* oviposition. Previous studies have shown that NSTs inhibit *F. fusca* oviposition in susceptible populations and fail to reduce oviposition in NST-resistant populations (Huseth et al. 2017). Further, NST efficacy has been shown to decrease over time as plants grow larger, allowing even NST-susceptible *F. fusca* to oviposit and establish larvae on older cotton seedlings (D'Ambrosio et al. 2018a). While crop plants rapidly take up and distribute NSTs systemically within their tissues, previous studies indicated that such compounds do not uniformly distribute within cotton seedlings but are instead primarily concentrated in the cotyledons (Elbert et al. 1998, Zhang et al. 2011). Depending on how *F. fusca* utilize the various structures of a cotton seedling for oviposition, this may affect the intensity of neonicotinoid exposure, possibly leading to resistance-favoring low-dose selection (Gressel 2011).

With this study, we sought to better understand *F. fusca* oviposition on NST-grown cotton. We examined oviposition patterns in both a greenhouse setting utilizing a neonicotinoid-naïve, laboratory-raised *F. fusca* population to gain baseline knowledge of *F. fusca* oviposition in the presence and absence of insecticidal compounds. We followed this with a field study involving a neonicotinoid-resistant *F. fusca* population to see if patterns and responses were conserved in the field in the presence of a NST-resistant population. Based on previous studies showing that NSTs decrease in efficacy as seedlings

grow older (D'Ambrosio et al. 2018a), we chose to examine oviposition behavior on seedlings of varying ages in both studies. Because thrips orient themselves relative to touch stimuli (thigmotaxis) (Kirk 1997) and damage is known to manifest at the growth point of cotton seedlings, affecting true leaf development and apical dominance (Hawkins et al. 1966), we hypothesized that *F. fusca* would oviposit in or near the emerging leaves on the seedling. Further, based on previous studies of NST performance against *F. fusca* over time (D'Ambrosio et al. 2018a), we hypothesized that the oviposition suppression effects of NSTs against both populations would decrease over time as seedlings became older and larger. Finally, based on previous observations of NST effects on oviposition (Huseth et al. 2017), we hypothesized that NSTs would reduce oviposition by the neonicotinoid-naïve population, but this effect would be less apparent in the resistant field population.

Methods

Cotton Seed

Cotton seedlings of the cultivar Stoneville 4946GLB2 (ST4946) were used in both experiments. Selection of this variety was based on its regional suitability for the field trial location (Stoneville Cotton Variety Overview 2018). ST4946 has herbicide tolerance traits for glyphosate and glufosinate-ammonium and expresses insecticidal proteins *Bacillus thuringiensis* Cry1Ac and Cry2Ab2. These traits are representative of traits that *F. fusca* would encounter in a commercial field setting, and none have any documented activity against *F. fusca*. All seeds were treated with metalaxyl, penflufen, prothioconazole, and mycobutanil (Allegence-FL, EverGol Prime, Proline 480SC, Bayer CropScience St. Louis, MO; Spera 240FS, Nufarm Agricultural Products, Alsip, IL, respectively) for pathogen management. NST seeds additionally received an insecticidal active ingredient (thiamethoxam or imidacloprid) at the labeled rate of 0.375 mg ai seed^{-1}.

Thrips

The neonicotinoid-naïve, laboratory-reared *F. fusca* population used in greenhouse studies was reared on white cabbage (*Brassica oleracea* var. *capitata*) leaves in a controlled environment of 26°C with ca. 60% RH and a photoperiod of 16:8 (L:D) h. For field studies, the resident *F. fusca* population was allowed to naturally infest seedlings. To determine the neonicotinoid resistance levels of these insects, randomly distributed plots of untreated cotton seedlings within the field trial were destructively harvested at the beginning of the season, returned to the laboratory, and allowed to dry down. Immature thrips that departed from these drying seedlings were offered leaves of white cabbage and reared on the cabbage to adult. A subset of adult females from each population was subject to a previously described, diet-based feeding bioassay (Huseth et al. 2016). Based on the methodology of Huseth et al. (2016), data were analyzed using logistic regression with the PROC GLIMMIX in the SAS system, version 9.3 (SAS Institute, Cary, NC). Surviving thrips at the end of the assay period were modeled as a binary outcome of the $\log(x + 1)$ of insecticide dosage. For each population, this produced a dose coefficient (slope), as well as an intercept, which were used to inversely calculate LC_{50} values (Huseth et al. 2016). Confidence intervals around the LC_{50} estimate were determined by using the confidence intervals of the slope estimate. The rearing of immature field thrips to adult also allowed for a determination of an approximate composition of thrips species directly infesting the cotton seedlings. *Frankliniella fusca* is visually distinct from other cotton-infesting thrips in North Carolina due to its dark coloration. This contrasts from other species, which are either lighter in color (e.g., other *Frankliniella* spp., *Thrips tabaci* (Lindeman)) or distinctly striped (e.g., *Neohydatothrips variabilis* (Beach)). Thrips reared from harvested seedlings in both years were visually estimated to be >95% *F. fusca*. A subset of visually identified, putative *F. fusca* was slide mounted and examined with a stereomicroscope to confirm species identity (Palmer et al. 1992).

Greenhouse Studies

Greenhouse studies took place at the Method Road Greenhouse facility on the campus of North Carolina State University in Raleigh, NC, in 2016. Experiments were conducted at 27 ± 3°C under a 16:8 (L:D) h light cycle. Cotton seeds were sown individually at a depth of 25 mm into 150-mm-diameter terra cotta pots containing a soil mixture of 2:2:1 potting mixture (Fafard, Agawam, MA), steam-sterilized loam, and steam-sterilized sand. Following the methods of D'Ambrosio et al. (2018a), seedlings were covered with an insect-proof enclosure constructed of a modified 2-liter beverage bottle and received water through an automated system delivering ca. 63.5 ml of water per pot over a 3-min interval every 6 h (total ca. 250 ml/d), thereby maintaining adequate soil moisture without any drainage of water or leaching of insecticide through the pot. To observe any age-related effects on NST efficacy, plants were grown to one of three ages before being infested by *F. fusca*: 9 days after planting (DAP; 0–1 true leaves), 14 DAP (2–3 true leaves), and 19 DAP (3–4 true leaves). These plant ages encompassed the period in which cotton seedlings are most susceptible to thrips injury, which is between emergence and 4–5 true leaves (Cook et al. 2011; Reisig 2014, 2016). Planting dates were staggered so that all plants could be infested with *F. fusca* at a single time point. To infest plants, five adult female *F. fusca* were aspirated into 1.5-ml microcentrifuge tubes, one of which was then placed into each insect-proof cage and opened to release the thrips. Insects were allowed access to the seedlings for 3 d, allowing enough time for oviposition but not egg hatching (Watts 1934, Lowry et al. 2014) before the seedlings were destructively sampled by clipping the hypocotyl. Ninety cotton seedlings were grown for the greenhouse experiment, allowing for 10 replicates of each insecticide treatment × seedling age combination.

Field Studies

Field trials were conducted at the North Carolina Department of Agriculture and Consumer Services' Upper Coastal Plain Research Station in Rocky Mount, NC, in 2016 and 2017 (35.8934°N, –77.6773°W). Seeds were machine planted in 12 m long, 4 row plots with 0.9 m row centers at a seeding rate of 14 seeds m^{-1}, for a stocking density of 143,518 seeds ha^{-1}. Plots were separated with 1.5 m of bare soil. Two seedlings were randomly selected from each plot, and destructively sampled by clipping the hypocotyl at ground level. To observe any time-related effects in NST efficacy, samples were conducted twice each field season, when seedlings had ca. 2 and 4 true leaves. Twenty-four seedlings in total were harvested each sampling day, allowing eight replicates of each insecticide treatment × seedling age combination in each field season. This resulted in a total of 16 replicates per insecticide treatment × seedling age combination across both field trial years.

To better understand *F. fusca* reproductive habits on cotton, a concurrent evaluation of larval distribution on field-grown seedlings was also made. In addition to samples collected for egg numbers, eight seedlings were randomly selected from each plot and divided by plant part (cotyledon or true leaf). Composite samples were placed into 260-ml polypropylene jars (#128070TSPP, Mold-Rite Plastics, Plattsburgh, NY) containing 150 ml of water and 250 µl detergent.

One composite sample, divided by plant part, was collected from each of the four experimental blocks each sampling day, resulting in a total of eight replicates per insecticide treatment × seedling age × plant part combination across both field trial years.

Seedling Processing

Clipped greenhouse and field seedlings were returned to the laboratory where they were cleared and stained with a previously described lactophenol, acid fuchsin clearing and staining technique (Riley et al. 2007). Briefly, seedlings were held in a 60°C heated bath of 2:1:1 95% EtOH, 10% lactic acid, glacial acetic acid for 4 min to clear leaves, and then rinsed and transferred into a boiling bath of 1:1:2:1:1 water, 10% lactic acid, 50% glycerol, phenol, and 1 g/liter acid fuchsin solution for 1 min to stain leaves. Stained plant material was spread onto 150-mm-diameter plastic Petri dishes (cat. no.: 25384-094, VWR, Radnor, PA), rinsed with tap water to remove excess staining solution, and allowed to dry. Eggs were counted by using a backlit stereomicroscope. The location of each egg (cotyledon or true leaf) was also recorded. To account for any impacts of variation in cotyledon/true leaf size in both experiments (i.e., small leaves having fewer eggs and vice versa), we additionally examined egg density as a response variable. Egg density was calculated by dividing the number of eggs by the leaf area. Leaf area was calculated by scanning the Petri dishes containing the spread leaves with an image scanner (DCP-7065 DN, Brother International, Bridgewater, NJ) to create digital image files. The files were analyzed using the GNU Image Manipulation Program version 2.8 (The GIMP Team 2016) to measure the area of the scanned leaves in pixels, which were converted to square centimeters. Density calculations were made for both cotyledons and true leaves for each seedling. Data on seedling leaf area can be found in the Supp Information (online only).

Larval distribution on seedlings from the field samples was determined by separately rinsing cotyledons and true leaves with water through a series of sieves (Dual Manufacturing Co., Inc., Franklin Park, IL). Samples were initially passed through a 500 micron (0.5 mm) mesh sieve to remove large pieces of debris, then through a finer, 150 micron (0.15 mm) mesh sieve to recover larvae (Rummel and Arnold 1989). Larvae were rinsed from the final sieve with 70% EtOH into 20 ml vials (#03-337-23, Fisher Scientific International Inc., Pittsburgh, PA), and counted with a stereomicroscope.

Statistical Analysis

Egg numbers, egg densities, and larval counts were transformed to $\log(x + 1)$ to meet assumptions of normality. Values were analyzed using PROC GLIMMIX in the SAS system, version 9.3, with a model containing main effects of insecticide treatment and leaf type, along with their interaction. For field-based studies, year was also included as a main effect to account for annual variation in thrips pressure and growing conditions. Experimental block was included as a random effect in all analyses. Analyses were conducted separately by plant age for both greenhouse and field studies.

Results

Frankliniella fusca Resistance Levels

The laboratory *F. fusca* population had LC_{50} values of 2.2 ppm (confidence limits [CL] 1.8–3.0 ppm) for thiamethoxam and 0.9 ppm (CL 0.8–1.2 ppm) for imidacloprid. The field *F. fusca* population had LC_{50} values of 204.4 ppm (89.9–664.3 ppm) for thiamethoxam and 30.9 ppm (CL 18.2–64.2 ppm) for imidacloprid in 2016, and 41.0 ppm (CL 22.8–94.7 ppm) for thiamethoxam and 26.5 ppm (CL 15.9–53.8 ppm) for imidacloprid in 2017.

Greenhouse Experiment

Egg counts

Significant effects of insecticide treatment, and a significant insecticide treatment × leaf type were seen at all plant ages. A significant effect of leaf type was seen at 9 and 14 DAP, but not at 19 DAP (Table 1). The greenhouse *F. fusca* population oviposited significantly more eggs into the fungicide-only treated seedlings at 9 (41.9 ± 9.1) and 14 DAP (30.4 ± 7.2) relative to the thiamethoxam treatment at 9 (12.7 ± 3.8) and 14 DAP (9.5 ± 2.6) and imidacloprid treatment at 9 (2.2 ± 0.5) and 14 DAP (3.6 ± 1.2). Similarly, at 19 DAP, more eggs were laid into fungicide-treated cotton (19.6 ± 3.7) than cotton treated with imidacloprid (4.7 ± 1.0). Egg distribution also differed among treatments, with significantly more eggs oviposited into cotyledons than true leaves at all plant ages in the fungicide-only treated seedlings, and more uniform egg distribution in the NST-grown seedlings at 14 and 19 DAP (Fig. 1).

Table 1. Type III effects for egg number and egg density in greenhouse experiments with a neonicotinoid-susceptible *F. fusca* population

Distribution	Plant age	Parameter	Num. df	Denom. df	*F*-value	Pr > *F*
Egg number	9 DAP (0–1 true leaves)	Insecticide treatment (IT)	2	38	24.65	<0.0001
		Leaf type (LT)	1	38	231.29	<0.0001
		IT × LT	2	38	24.61	<0.0001
	14 DAP (2–3 true leaves)	Insecticide treatment (IT)	2	54	14.54	<0.0001
		Leaf type (LT)	1	54	17.71	0.0004
		IT × LT	2	53	7.37	0.0015
	19 DAP (3–4 true leaves)	Insecticide treatment (IT)	2	54	16.85	<0.0001
		Leaf type (LT)	1	54	0.33	0.5653
		IT × LT	2	54	8.85	0.0005
Egg density	9 DAP (0–1 true leaves)	Insecticide treatment (IT)	2	38	33.20	<0.0001
		Leaf type (LT)	1	38	92.18	<0.0001
		IT × LT	2	38	28.16	<0.0001
	14 DAP (2–3 true leaves)	Insecticide treatment (IT)	2	51.78	17.25	<0.0001
		Leaf type (LT)	1	53	14.50	0.0004
		IT × LT	2	53	17.08	<0.0001
	19 DAP (3–4 true leaves)	Insecticide treatment (IT)	2	54	11.16	<0.0001
		Leaf type (LT)	1	54	0.41	0.5226
		IT × LT	2	54	10.32	0.0002

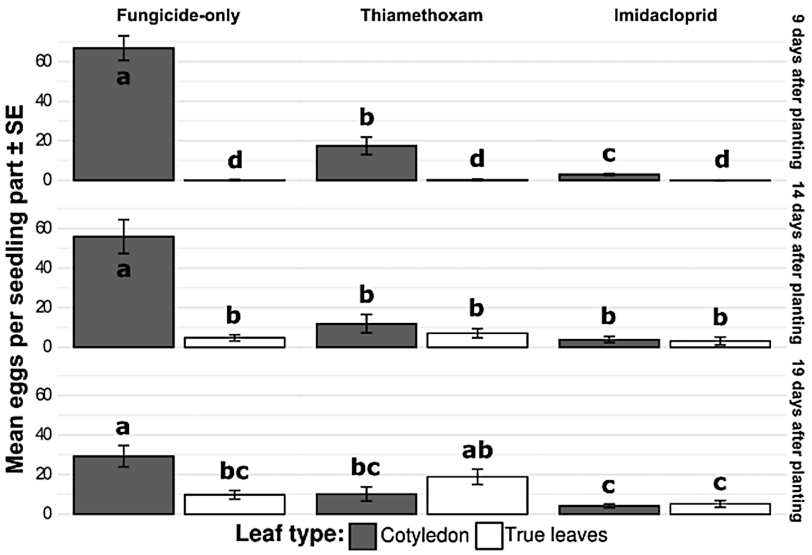

Fig. 1. Mean *Frankliniella fusca* eggs per seedling by leaf type with standard error in the greenhouse study involving a neonicotinoid-susceptible laboratory population. Bars with the same letters within DAP interval do not significantly differ. Means separations by Tukey's HSD at $\alpha = 0.05$.

Egg density

Analyses of density showed significant effects of insecticide treatment, and a significant insecticide treatment × leaf type at all plant ages. A significant effect of leaf type was seen at 9 and 14 DAP, but not at 19 DAP (Table 1). Density patterns were similar to those observed with egg numbers (Fig. 2). However, a significantly higher density of eggs was observed on the true leaves of thiamethoxam-treated plants at 19 DAP relative to those in the cotyledons of these same plants (Fig. 2), which was not observed in the egg number analysis (Fig. 1).

Field Experiments

Egg counts

Significant effects of leaf type and trial year (higher egg numbers in 2016) were seen at both sample dates. Neither a significant insecticide treatment × leaf type interaction, nor an effect of insecticide treatment, was seen in either sample (Table 2). Egg distributions between cotyledons and true leaves did not differ among treatments at the first sample (Fig. 3). At the second sample, significantly more eggs were found in cotyledons relative to true leaves on the fungicide-only treated plants, with no statistical difference between cotyledons and true leaves in the thiamethoxam- and imidacloprid-treated plants (Fig. 3).

Egg density

Significant effects of leaf type were seen at both sample dates. Significant effects of insecticide treatment were seen in the second sample, but not in the first. Neither a significant insecticide treatment × leaf type interaction, nor an effect of trial year, was seen in either sample (Table 2). Means separation patterns were identical to those observed for egg numbers (Fig. 4).

Larval counts

Significant effects of insecticide treatment, leaf type, and trial year (higher larval numbers in 2017) were seen in both samples. No significant insecticide treatment × leaf type interaction was seen in either sample (Table 2). Larvae were uniformly distributed between cotyledons and true leaves in all treatments on both sample dates except for thiamethoxam on the first sample date, where significantly more larvae were found on true leaves (Fig. 5).

Discussion

Greenhouse Findings

As previously reported (Huseth et al. 2017), NSTs reduced oviposition relative to untreated plants, which is expected given the resistance background of the laboratory-raised *F. fusca* population (Fig. 1). This effect also decreased over time, reflecting a decline in the residual efficacy of the treatments. Similar findings have been observed in studies examining larval numbers over time on NST cotton (D'Ambrosio et al. 2018a). Within treatment, there is also a difference in egg numbers between cotyledons and true leaves. At 14 and 19 DAP, egg numbers were uniform between cotyledons and true leaves in the imidacloprid- and thiamethoxam-treated seedlings. In the fungicide-only plants, significantly more eggs were still found in the cotyledons. At 9 DAP, however, significantly more eggs were found in cotyledons relative to true leaves in all treatments (Fig. 1). This is likely due to the true leaves being very small relative to the cotyledons at this age. Examining egg density distribution patterns can help to account for these small leaves by correcting for the size disparity.

Analyses of egg density showed that in untreated plants, *F. fusca* preferentially oviposited into cotyledon tissue. This was seen at every plant age observed. NSTs altered this preference, however, with more equal, albeit lower, densities of eggs seen in both tissue types (Fig. 2). Given that NSTs distribute within the cotyledons of cotton seedlings at higher levels than in true leaves (Elbert et al. 1998, Zhang et al. 2011), this finding suggests that *F. fusca* alter their oviposition behavior in response to the insecticidal compounds and select an alternative leaf type for oviposition where insecticide exposure is lower. As plants grow older, more true leaf area is available for oviposition. While egg density in true leaves increased with plant age in untreated and treated plants alike as a result, the effect was more pronounced in treated plants, with densities in true leaves equal to or greater than those observed in cotyledons on the oldest seedlings (Fig. 2). Aging cotyledons becoming less preferable for oviposition could also cause some of the oviposition shift to true leaves in older plants, but

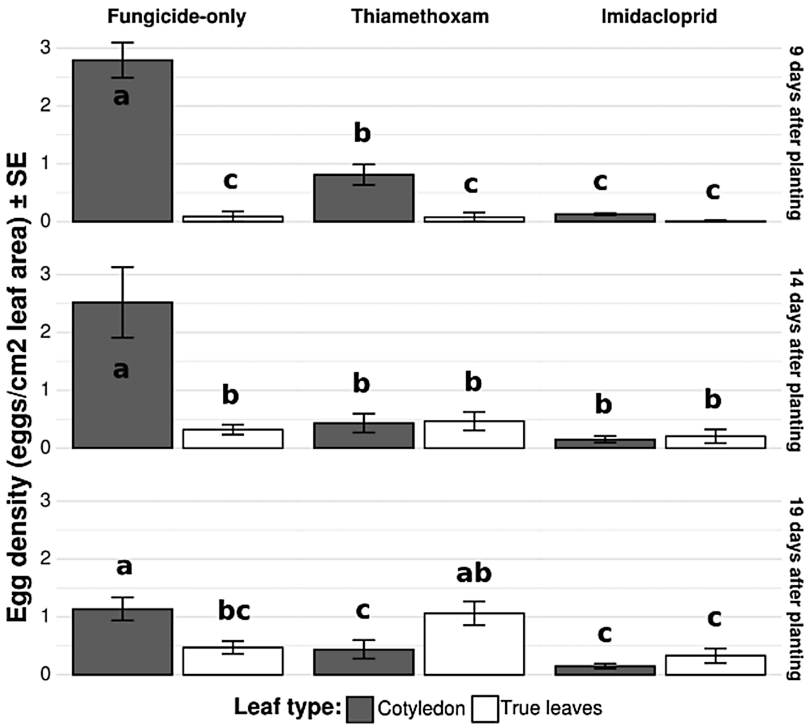

Fig. 2. *Frankliniella fusca* egg density with standard error in the greenhouse study involving a neonicotinoid-susceptible laboratory population. Bars with the same letters within DAP interval do not significantly differ. Means separations by Tukey's HSD at $\alpha = 0.05$.

Table 2. Type III effects for egg number, egg density, density and larval distribution in field experiment involving a neonicotinoid-resistant *F. fusca* population

Distribution	Sample (seedling development)	Parameter	Num. df	Denom. df	*F*- value	Pr > *F*
Egg numbers	1 (ca. 2 true leaves)	Insecticide treatment (IT)	2	104	0.05	0.9500
		Leaf type (LT)	1	104	425.76	<0.0001
		IT × LT	2	104	2.13	0.1243
		Trial year	1	104	4.86	0.0296
	2 (ca. 4 true leaves)	Insecticide treatment (IT)	2	84.78	1.79	0.1732
		Leaf type (LT)	1	65.71	23.31	<0.0001
		IT × LT	2	84.78	0.06	0.9447
		Trial year	1	65.71	4.15	0.0456
Egg density	1 (ca. 2 true leaves)	Insecticide treatment (IT)	2	104	1.64	0.1995
		Leaf type (LT)	1	104	204.15	<0.0001
		IT × LT	2	104	2.46	0.0907
		Trial year	1	104	0.29	0.5889
	2 (ca. 4 true leaves)	Insecticide treatment (IT)	2	87	5.96	0.0038
		Leaf type (LT)	1	87	27.80	<0.0001
		IT × LT	2	87	0.55	0.5775
		Trial year	1	87	<0.01	0.9528
Larvae	1 (ca. 2 true leaves)	Insecticide treatment (IT)	2	46	16.26	<0.0001
		Leaf type (LT)	1	46	11.52	0.0014
		IT × LT	2	46	3.05	0.0568
		Trial year	1	46	108.08	<0.0001
	2 (ca. 4 true leaves)	Insecticide treatment (IT)	2	49	4.35	0.0182
		Leaf type (LT)	1	49	15.11	0.0003
		IT × LT	2	49	0.35	0.7088
		Trial year	1	49	19.35	<0.0001

the stark contrast in oviposition patterns observed between older untreated and NST-grown seedlings suggests insecticide presence in the treated cotyledons is a much greater factor in driving this shift. These results show that *F. fusca* demonstrate a clear preference for oviposition into cotyledon tissue, but alter this behavior in response to systemic neonicotinoids, resulting in greater oviposition elsewhere on the plant. This not only reduces larval exposure to lethal levels of insecticides, but, by allowing them to access an insecticide-treated plant as a reproductive resource, may enhance selection for resistance through low-dose exposure (Gressel 2011).

Field Findings—Oviposition

Unlike patterns observed in the greenhouse, insecticide treatment did not reduce egg numbers in the field (Fig. 3). This is reflective of the

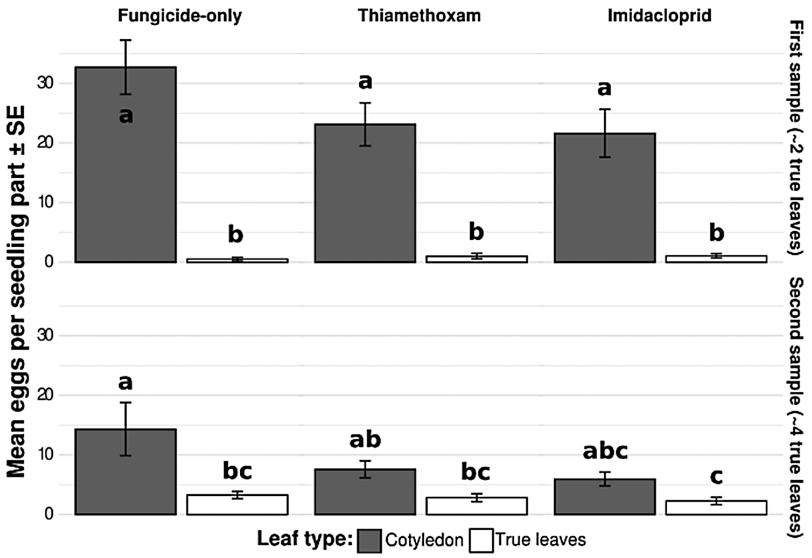

Fig. 3. *Frankliniella fusca* eggs per seedling by leaf type with standard error in the field study involving a neonicotinoid-resistant population. Bars with the same letters within sample do not significantly differ. Means separations by Tukey's HSD at $\alpha = 0.05$.

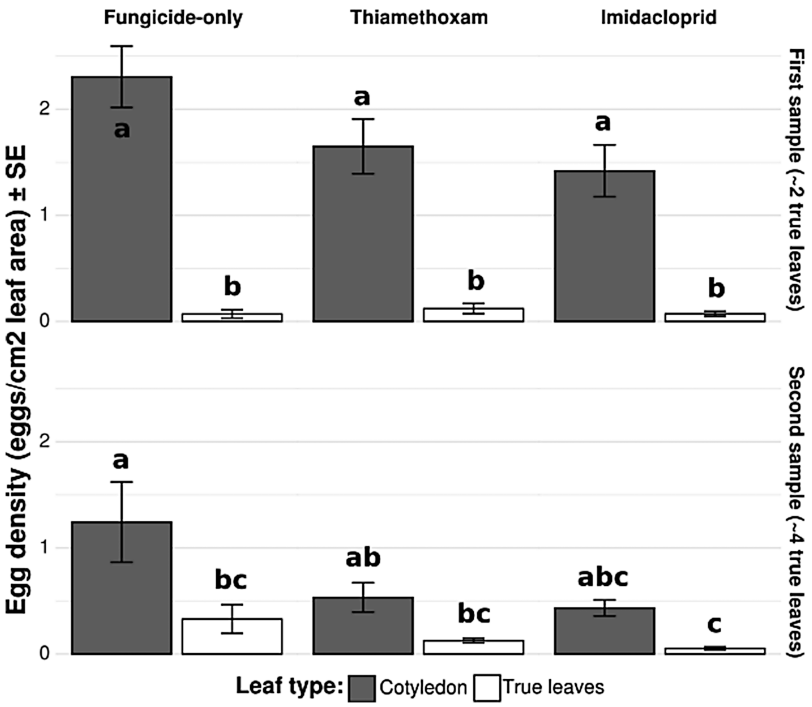

Fig. 4. *Frankliniella fusca* egg density with standard error in the field study involving a neonicotinoid-resistant population. Bars with the same letters within sample do not significantly differ. Means separations by Tukey's HSD at $\alpha = 0.05$.

resistant background of the field population, which had LC_{50} values that were at least 18.6 and 29.4× higher than the laboratory population for thiamethoxam and imidacloprid, respectively. This loss of NST-driven oviposition deterrence against resistant populations has been observed in previous laboratory studies as well (Huseth et al. 2017). Egg number distributions between cotyledons and true leaves did not differ among treatments at the first sample period. Egg numbers were significantly higher on cotyledons relative to true leaves on the fungicide-only treatment at the second sample date and did not differ between cotyledons and true leaves in the imidacloprid- and thiamethoxam-treated plants (Fig. 3).

Analyses of egg density demonstrated that similar to the patterns seen in the greenhouse, *F. fusca* in the field showed a preference for cotyledon tissue when ovipositing (Fig. 4). This similarity shows that cotyledon preference is not an artifact of a controlled greenhouse setting but is instead conserved in the field where other factors, such as precipitation, could influence oviposition site selection. Unlike the laboratory population, the effect of treatment was less apparent, with these insects ovipositing into NST-treated cotyledons at levels similar to those in untreated cotyledons (Figs. 3 and 4). Together these results show that resistant thrips do not display strong behavioral avoidance to these compounds and instead utilize

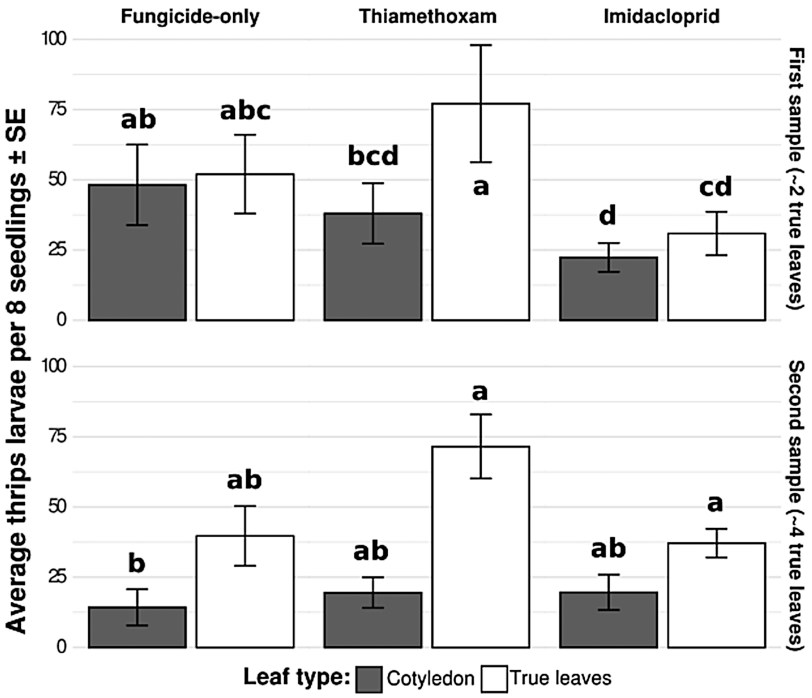

Fig. 5. *Frankliniella fusca* larval numbers with standard error in the field study involving a neonicotinoid-resistant population. Bars with the same letters within sample do not significantly differ. Means separations by Tukey's HSD at $\alpha = 0.05$.

the preferred cotyledon tissue for oviposition on insecticide-treated plants. Egg numbers and density alike did not significantly differ on the cotyledons versus true leaves in the imidacloprid- and thiamethoxam-treated plants at the second sample date (Figs. 3 and 4). This is similar to patterns observed in the greenhouse on older, treated plants (Figs. 1 and 2). However, mean numbers of thrips eggs were higher on cotyledons than true leaves in the field population (Fig. 1), but were higher on true leaves than cotyledons in the greenhouse population (Fig. 3). While this may be indicative of some behavioral avoidance in the field, it may also reflect heterogeneity in the field population, with the more resistant individuals ovipositing on the preferred cotyledons, and the more susceptible individuals ovipositing on true leaves to avoid the treated cotyledon tissue. This is further supported by the large confidence intervals around the LC_{50} estimates in this population reported earlier. If these experiments were repeated with a more resistant and more homogeneous population, it is likely that significantly more eggs would be recovered on treated cotyledons than true leaves at the second sample date.

Field Findings—Larval Distribution

The results of both the greenhouse and field oviposition data seemingly conflict with observations of thrips injury in the field, which are primarily seen at the growth point and young true leaves (Hawkins et al. 1966). Initially, this suggested that thrips larvae hatch primarily on the cotyledons of cotton seedlings, then move toward the growth point where they feed on developing foliage. Our concurrent evaluation of larval distribution in the field supported this idea.

Unlike eggs, larvae were recovered in equal or greater numbers on true leaf tissue relative to cotyledon tissue. These patterns were seen in all treatments (Fig. 5). Given this population's strong preference for cotyledon tissue as an oviposition substrate on plants in these same plots, especially at the first sample date (Fig. 4), this showed that while eggs are primarily oviposited onto cotyledons, larvae tended to distribute themselves more uniformly throughout the plant. This would allow them to injure developing true leaves as well as the growth point of seedlings, thereby causing the injury patterns commonly observed in cotton fields afflicted by *F. fusca*.

Implications for *F. fusca* Control and Resistance Management

These findings provide a biological rationale to current thrips control tactics and offer a foundation for future insecticide resistance management (IRM) tactics. NSTs tend to concentrate primarily in the cotyledons of cotton seedlings, with lower concentrations present in newer true leaves (Elbert et al. 1998, Zhang et al. 2011). This explains why these products, until recently, were very effective in thrips management, as the highest insecticide concentrations were present at the area of the plant most utilized by *F. fusca* for oviposition. By altering their oviposition behavior, however, *F. fusca* can use insecticide-treated plants as a reproductive resource, in turn potentially exposing larvae to low levels of insecticides. Over time, this low-dose selection could promote an increase in the frequency of resistant individuals in a population and lead to widespread resistance to these compounds (Gressel 2011). In areas where NSTs are failing, growers are currently advised to make foliar sprays of insecticides such as acephate (Reisig 2018), with the recommendation that sprays be targeted around the emergence of the first true leaf (Collins et al. 2015, Reisig 2015). Our findings offer a biological basis as to why these early sprays work, as they are reducing populations of larvae emerging primarily on the cotyledons before they can disperse to the developing true leaves. In lieu of NSTs, such foliar sprays have utility in controlling neonicotinoid-resistant *F. fusca*, provided they are timed to kill larval thrips hatching on the cotyledons and before they can damage the young true leaves (D'Ambrosio et al. 2018b). Insecticide foliar sprays with different modes of action, such as spinetoram (Insecticide Resistance Action Committee [IRAC] group 5) and cyantraniliprole (IRAC group 28) (IRAC Mode of Action Classification 2018), are registered for use on cotton, and have

demonstrated activity against *F. fusca*, including NST-resistant populations (Huseth et al. 2017, D'Ambrosio et al. 2018b). If applied in a timely fashion, such products can be effective in controlling *F. fusca*, and may improve IRM tactics by offering alternative modes of action for thrips management in cotton.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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References Cited

Collins, G., D. D. Reisig, K. Edmisten, and A. York. 2015. It's time to evaluate emerged cotton for thrips and other factors. https://cotton.ces.ncsu.edu/2015/05/its-time-to-evaluate-emerged-cotton-for-thrips-and-other-factors-collins-reisig-edmisten-york/ [accessed 16 April 2018].

Cook, D., A. Herbert, D. S. Akin, and J. Reed. 2011. Biology, crop injury, and management of thrips (Thysanoptera: Thripidae) infesting cotton seedlings in the United States. J. Int. Pest Manage. 2: B1–B9.

D'Ambrosio, D. A., A. S. Huseth, and G. G. Kennedy. 2018a. Temporal efficacy of neonicotinoid seed treatments against *Frankliniella fusca* on cotton. Pest Manag. Sci. 113: 56–63.

D'Ambrosio, D. A., A. S. Huseth, and G. G. Kennedy. 2018b. Evaluation of alternative mode of action insecticides in managing neonicotinoid-resistant *Frankliniella fusca* in cotton. Crop Prot. 113: 56–63.

Elbert, A., R. Nauen, and W. Leicht. 1998. Imidacloprid, a novel chloronicotinyl insecticide: biological activity and agricultural importance, pp. 50–73. *In* I. Ishaaya and D. Degheele (eds.), Insecticides with novel modes of action. Springer Berlin, Heidelberg, Berlin.

Elbert, A., M. Haas, B. Springer, W. Thielert, and R. Nauen. 2008. Applied aspects of neonicotinoid uses in crop protection. Pest Manag. Sci. 64: 1099–1105.

Gaines, J. C. 1934. A preliminary study of thrips on seedling cotton with special reference to the population, migration, and injury. J. Econ. Entomol. 27: 740–743.

Gressel, J. 2011. Low pesticide rates may hasten the evolution of resistance by increasing mutation frequencies. Pest Manag. Sci. 67: 253–257.

Hawkins, B. S., H. A. Peacock, and T. E. Steele. 1966. Thrips injury to upland cotton (*Gossypium hirsutum* L.) varieties. Crop Sci. 6: 256–258.

Huseth, A. S., T. M. Chappell, K. Langdon, S. C. Morsello, S. Martin, J. K. Greene, A. Herbert, A. L. Jacobson, F. P. Reay-Jones, T. Reed, et al. 2016. *Frankliniella fusca* resistance to neonicotinoid insecticides: an emerging challenge for cotton pest management in the eastern United States. Pest Manag. Sci. 72: 1934–1945.

Huseth, A. S., D. A. D'Ambrosio, and G. G. Kennedy. 2017. Responses of neonicotinoid resistant and susceptible *Frankliniella fusca* life stages to multiple insecticide groups in cotton. Pest Manag. Sci. 73: 2118–2130.

Kirk, W. D. J. 1997. Distribution, abundance, and population dynamics, pp. 257–271. *In* T. Lewis (ed.), Thrips as crop pests. Centre for Agriculture and Bioscience International, Wallingford, Oxfordshire, United Kingdom.

Lowry, V. K., J. W. Smith, Jr., and F. L. Mitchell. 2014. Life-fertility tables for *Frankliniella fusca* (Hinds) and *F. occidentalis* (Pergrande) (Thysanoptera: Thripidae) on peanut. Ann. Entomol. Soc. Am. 85: 744–754.

Palmer, J. M., L. A. Mound, and G. J. Du Haeume. 1992. IIE guides to insects of importance to man, vol. 2. Thysanoptera. C. R. Bets (ed.). International Institute of Entomology, CAB International, Wallingford, Oxfordshire, United Kingdom.

Reisig, D. D. 2014. Thrips are punishing cotton. https://cotton.ces.ncsu.edu/2014/05/thrips-are-punishing-cotton/ [accessed 16 April 2018].

Reisig, D. D. 2015. Should you spray for cotton thrips? https://cotton.ces.ncsu.edu/2015/05/should-you-spray-for-cotton-thrips/ [accessed 16 April 2018].

Reisig, D. D. 2016. Managing insects on cotton. https://content.ces.ncsu.edu/cotton-information [accessed 20 February 2017].

Reisig, D. D. 2018. Managing insects on cotton. 2018 Cotton Information. https://content.ces.ncsu.edu/cotton-information [accessed 30 April 2018].

Riley, D. G., A. Chitturi, and A. N. Sparks, Jr. 2007. Does natural deposition of pine pollen affect the ovipositional behavior of *Frankliniella occidentalis* and *Frankliniella fusca*? Entomol. Exp. Appl. 124: 133–141.

Rummel, D. R., and M. D. Arnold. 1989. Estimating thrips populations in cotton with conventional sampling and a plant washing technique. Southwest. Entomol. 14: 279–285.

Stoneville Cotton Variety Overview. 2018. https://www.cropscience.bayer.us/products/seeds/stoneville-cotton/variety-overview [accessed 15 February 2018].

The GIMP Team. 2016. GIMP: the GNU image manipulation program. https://www.gimp.org.

The IRAC Mode of Action Classification. 2018. Insecticide resistance action committee. http://www.irac-online.org/modes-of-action/ [accessed 12 April 2018].

Watts, J. G. 1934. A comparison of the life cycles of *Frankliniella tritici* (Fitch), *F. fusca* (Hinds) and *Thrips tabaci* (Lind.) (Thysanoptera-Thripidae) in South Carolina. J. Econ. Entomol. 27: 1158–1159.

Zhang, L., S. M. Greenberg, Y. Zhang, and T. X. Liu. 2011. Effectiveness of thiamethoxam and imidacloprid seed treatments against *Bemisia tabaci* (Hemiptera: Aleyrodidae) on cotton. Pest Manag. Sci. 67: 226–232.