D’AMBROSIO, DAMON ANGELO. Evaluation of Multiple Reduced Risk Chemistries on Thrips Feeding and Oviposition in Cotton and Tomato. (Under the direction of Dr. George G. Kennedy).

Thrips (Thysanoptera: Thripidae) serve as both indirect and direct pests in a variety of cropping systems. Directly, thrips feeding and oviposition can injure developing plants, and reduce the quality and marketability of various crops. Indirectly, thrips can serve as vectors for economically damaging plant viruses such as *Tomato spotted wilt orthotospovirus* (TSWV), which they transmit through feeding. Insecticides serve as important management tools for these insects. Understanding the effect of insecticides on injurious aspects of thrips behavior can increase the understanding of how such behavioral effects ultimately reduce injury. In instances of resistance, applied behavioral studies can provide insight into how thrips overcome insecticides, informing future management efforts. I evaluated injurious thrips behavior and the effect of insecticides in two agroecosystems: tomato and cotton.

In North Carolina and much of the southeastern USA, *Frankliniella fusca* and *F. occidentalis* are the primary thrips pests of tomato. Both are important vectors of TSWV. Systemic insecticides are often applied to tomato for protection. An electrical penetration graphing (EPG) study was conducted to determine the feeding behavior of these two vectors on tomato, and observe how the behaviors are affected by two systemic insecticides: imidacloprid and cyantraniliprole. Previous studies have identified the feeding behaviors most conducive to successful virus transmission. I built on this to deduce how insecticides ultimately impact virus transmission through feeding behavior modulation, and determined that imidacloprid and cyantraniliprole ultimately affect *F. fusca* and *F. occidentalis* feeding behavior differently to reduce the odds of transmission. Between species, cyantraniliprole and imidacloprid reduce
probing in *F. fusca*, but only imidacloprid consistently reduces probing in *F. occidentalis*. Within species, imidacloprid greatly reduces probing frequency in *F. fusca*. Cyantraniliprole confers fewer reductions in probing, but the feeding events that occur on these plants are less conducive to transmission. These results demonstrate that different insecticides can modulate behavior differently to ultimately reduce transmission.

In cotton, *F. fusca* serves as a direct pest of seedlings. Adult insects infest cotton fields, ovipositing into seedlings and exposing them to injurious larvae. Neonicotinoid seed treatments (NSTs) are widely used, and have been the mainstay in *F. fusca* management for nearly 20 years. Recently, NST resistance has been reported in multiple populations throughout the Southeast USA. I conducted multiple studies to better understand the *F. fusca*-NST interaction in cotton, and compared NST-susceptible and resistant *F. fusca* populations. I examined the temporal performance of NSTs to determine if their efficacy decreases as plants become larger and the effective concentration of neonicotinoids within the plants putatively decreases. Neonicotinoid susceptible and resistant populations were found to establish more larvae on older plants, but the resistant population can exploit this decrease in NST efficacy more rapidly. I also examined *F. fusca* oviposition and feeding behaviors using EPG on NST-grown cotton. I discovered that *F. fusca* preferentially oviposits on cotton cotyledons, but will oviposit into true leaves on NST-grown seedlings. This correlates with how neonicotinoids are reported to distribute in cotton seedlings, wherein concentrations are higher in the cotyledons. Resistant *F. fusca* do not avoid NST-treated cotyledons. The oviposition shift in susceptible *F. fusca* could allow them access to insecticide-treated plants as an oviposition resource, possibly encouraging low dose exposure and selecting for resistance. NSTs reduce feeding frequency in susceptible *F.
fusca populations, but not resistant *F. fusca*. Finally, I used the knowledge gained through EPG and greenhouse studies on the NST-*F. fusca* interaction to evaluate alternative mode of action foliar insecticide sprays for control of resistant *F. fusca* in a field trial setting, where I determined that foliar sprays can exceed the performance of NSTs against resistant insects.
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Evaluation of Multiple Reduced Risk Chemistries on Thrips Feeding and Oviposition in Cotton and Tomato

by
Damon Angelo D’Ambrosio

A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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DEDICATION

To my wife, Jodi: You’ve been by my side the whole way, took the brunt of countless bouts of venting about my research projects, and supported me throughout. I am excited to begin a chapter of our lives where neither of us are full-time students.

To my parents, Damon and Diane: You all let me bring jars of arthropods into the house as a kid (except for black widow spiders), which kicked off my interest in entomology. It looks like I will try and make a career out of it now.
BIOGRAPHY

Damon Angelo D’Ambrosio was born in Raleigh, NC, and was raised in nearby Fuquay-Varina, NC. As a child, Damon always had a fondness for arthropods, often attempting, with mixed success, to rear them in captivity. Growing up in Fuquay before the height of the Raleigh-metro population boom, Damon was exposed to many facets of commercial agriculture which, coupled with his family always having a vegetable garden, fostered his interest in agriculture. Damon graduated from Fuquay-Varina High School in 2008, and began studies at North Carolina State University with the goal of becoming a veterinarian. In his first semester, he learned that North Carolina State had an entomology program and decided to pursue a minor in the subject. His interest in entomology quickly surpassed his interest in veterinary medicine, and he changed his focus with the goal of becoming an entomologist. In the summer of 2010, Damon began working in Dr. George Kennedy’s program as an undergraduate assistant, where he was exposed to applied agricultural entomology and learned of career opportunities for entomologists in the agrochemical industry. Seeing an opportunity to meld his childhood interests into a viable career path, Damon graduated from North Carolina State University in 2012 with a BS in Zoology, and began his MS in Entomology under Dr. George Kennedy the following semester. In 2015, Damon was awarded the BASF Graduate Entomology Fellowship, allowing him to bypass his MS and pursue a PhD. With the BASF partnership, Damon was able to ensure the skills he obtained through his research would make him a viable and attractive candidate for employment in the agrochemical industry.
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INTRODUCTION

In the Southeastern United States, thrips (Thysanoptera: Thripidae) serve as both direct and indirect crop pests. While multiple strategies have been investigated to control thrips in various cropping systems, insecticides remain a major and important tool in their management. These insecticides can be delivered topically as foliar sprays, or systemically through transplant water, in-furrow application, tray drenches, or seed treatments (McPherson et al., 2003, Cook et al., 2011). By studying the behavior of thrips in particular agronomic settings, we can gain better insight on how these insects cause economic injury. While an insecticide’s mode of action provides insight into the molecular mechanisms that underlie its efficacy against thrips, studying how insecticides alter particular thrips behaviors provides additional insight into “how” a particular insecticide ultimately reduces thrips injury. Further, if resistance becomes an issue in a particular system and insecticides begin to fail, studying the behavior of resistant insects alongside their susceptible cohorts may provide valuable insight into why the insecticide failed, if behavioral modulation is a potential mechanism of resistance, and what measures can be taken in the future to more sustainably combat these insects. To date, behavioral mechanisms of resistance have not been identified in thrips. However, this is thought to be in large part due to thrips behaviors in response to insecticides being insufficiently studied (Gao et al., 2012, Jensen 2000).

As indirect pests, thrips transmit a suite of economically damaging plant viruses. Among these, *Tomato spotted wilt orthotospovirus* (TSWV), is the most economically damaging in the Southeast United States. Tomato spotted wilt (TSW) symptoms can vary by host (Best 1968), but in most cases, plants are killed and/or yield/marketability is reduced. Important TSW-
susceptible crops in the Southeast include peanuts (*Arapis hypogaea* L.), tobacco (*Nicotiana tabacum* L.), and vegetables such as tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.). In Georgia, estimates of economic loss in these crops were $32.6 million per year from 1996-2006, with nationwide losses extrapolated from this data estimated at $140 million per year (Riley et al., 2011). Globally, TSW has been estimated to cause economic losses of over $1 billion per year (Prins and Goldbach 1998). As vectors of TSWV, the behavior most pertinent to the thrips’ status as an indirect pest is their feeding, as virus is passed to plants via thrips salivation (Kindt 2004, Wijkamp et al., 1993). Previous studies have documented aspects of feeding behaviors that are associated with successful virus transmission (Kindt 2004, Stafford et al., 2011). However, few studies have documented the effects of insecticides on thrips feeding, especially focusing on behaviors specifically related to successful virus transmission (Jacobson and Kennedy 2013, Groves et al., 2001). Herein, I present a study documenting the effects of two systemic insecticides, imidacloprid and cyantraniliprole, on the feeding behavior of two economically important TSWV vectors: *Frankliniella fusca* (Hinds) and *F. occidentalis* (Pergande), on tomato (Chapter 1).

As direct pests, injury from adult and larval thrips feeding reduces the profitability of a wide variety of crops. In cotton (*Gossypium hirsutum* L.), *F. fusca* feeding on young seedlings can stunt or deform young plants, reduce the stress tolerance of the plant later in the season, making it more susceptible to stressors such as drought, and kill seedlings outright (Cook et al., 2011). Neonicotinoid seed treatments (NSTs) have been used for nearly 20 years to effectively manage early season injury on cotton seedlings from *F. fusca*, with adoption rates increasing throughout this time period. Currently, almost all cotton planted in the Southeast and Mid-
South is treated with a NST (North et al., 2018). Reports of NST field failures in recent years motivated a large scale survey of *F. fusca* populations throughout the Southeast and Mid-South, which discovered widespread resistance to these insecticides (Huseth et al., 2016). Previous studies of insecticide efficacy against *F. fusca* on cotton often utilize visual injury ratings or larval thrips counts (e.g. Herbert 1996, Copeland et al., 2016). While these metrics provide evidence of insecticide activity, their resolution is coarse, leaving many facets of the adult thrips interaction with the seedling and oviposition unknown. Recently a series of finer-resolution laboratory studies by Huseth et al. (2017) provided new insight on this interaction by examining the effects of NSTs on multiple life stages of both NST-susceptible and resistant *F. fusca*. Their findings revealed that NSTs have limited direct lethal activity against susceptible and resistant adult *F. fusca* alike. However, NSTs reduce oviposition in susceptible *F. fusca*, but fail to do so against resistant *F. fusca*. Based on their findings, I conducted a series of laboratory and greenhouse studies to further evaluate the effects of NSTs and resistance status on thrips larval establishment (Chapter 2), oviposition (Chapter 3) and feeding behavior (Chapter 4). From there, I used the knowledge gained on *F. fusca* interactions with NSTs in cotton to assess the potential of multiple, alternative mode of action foliar spray insecticides as management tools against resistant *F. fusca* in cotton in a small plot, field trial setting (Chapter 5).
REFERENCES


CHAPTER 1

Effects of systemic imidacloprid and cyantraniliprole on *Frankliniella fusca* and *F. occidentalis* feeding behavior on tomato

ABSTRACT

**BACKGROUND:** *Tomato spotted wilt orthotospovirus* (TSWV) is an economically-damaging plant disease vectored exclusively by thrips. In the Southeastern United States, the western flower thrips (*Frankliniella occidentalis*) and tobacco thrips (*F. fusca*) are the principal vectors responsible for its transmission in tomato. The objective of this study was to examine how two systemic insecticides, imidacloprid and cyantraniliprole, impact the feeding behaviors of these species that are conducive to virus transmission.

**METHODS:** Electrical penetration graphing was used to record probing and ingestion behaviors of both thrips species. Behaviors were recorded on imidacloprid- and cyantraniliprole-treated tomato plants, alongside insecticide-free control plants at 2-, 6-, and 10-days after treatment (DAT).

**RESULTS:** Relative to untreated plants, imidacloprid reduced the frequency of probing by both species. Cyantraniliprole reduced probing by *F. fusca* at all DAT intervals and *F. occidentalis* probing at 10 DAT only. Both species ingested more on cyantraniliprole-treated plants at some DAT intervals. In *F. fusca*, overall patterns of probing and ingestion on cyantraniliprole-treated plants were characteristic of those reported to be less conducive to virus transmission. Both insecticides likely have utility in controlling early-season primary virus spread by *F. fusca*, which
could have downstream effects on \textit{F. occidentalis} secondary virus spread later in the season by reducing available primary inoculum.

1 INTRODUCTION

In much of the southeastern United States, the economically-damaging \textit{Tomato spotted wilt orthotospovirus} (TSWV) is vectored in tomato agroecosystems by a complex of two thrips species: the tobacco thrips, \textit{Frankliniella fusca} (Hinds) and the western flower thrips, \textit{Frankliniella occidentalis} (Pergande) (Eckel et al., 1995, Riley and Pappu 2000, Riley and Pappu 2004, Salguero Navas et al., 1991, Toapanta et al., 1996, Weeks et al., 1990). \textit{Frankliniella fusca} is generally responsible for the early season, primary spread of the virus from overwintering weed hosts to tomato fields (Groves et al., 2001b, Groves et al., 2002, Joost and Riley 2004), and \textit{F. occidentalis} is responsible for later, in-season, secondary spread from tomato to tomato (Eckel et al., 1995, Joost and Riley 2004).

Although alternate control strategies such as reflective mulches and resistant cultivars have been employed to suppress tomato spotted wilt (TSW), insecticides remain an important tool in managing this disease (Riley and Pappu, 2004). Systemic formulations of insecticides such as imidacloprid and cyantraniliprole can be administered to tomato transplants to confer systemic protection from thrips and suppression of TSW (Groves et al., 2001a, Joost and Riley 2005, Riley and Pappu 2000, Riley and Pappu 2004, Bielza and Guillon, 2014, Jacobson and Kennedy 2011, Jacobson and Kennedy, 2013). Although their efficacy in suppressing TSW has been demonstrated by greenhouse and field trials (Groves et al., 2001a, Jacobson and Kennedy 2011, Jacobson and Kennedy 2013), little work has been done examining the insecticides’ effects on thrips feeding behavior (Groves et al., 2001a, Jacobson and Kennedy 2013, Joost and
The feeding behavior work done has had limited focus on behavior in the context of specific feeding behaviors conducive to virus transmission.

Thrips transmit TSWV through feeding. After a viruliferous thrips penetrates a plant and before it begins to ingest, virus particles are egested along with thrips saliva (Kindt 2004, Wijkamp 1993). However, once the thrips begins to ingest, it may re-ingest previously egested virus particles, reducing the amount of virus placed in the plant (Kindt 2004). Further, sustained periods of ingestion can damage cells in the area of feeding, which can inhibit the ability for TSWV to successfully infect the plant (Kindt 2004, Stafford et al., 2011, van de Wetering et al., 1998). Therefore, feeding behaviors most conducive to successful TSWV transmission involve many probes and short bouts of ingestion (Kindt 2004, Stafford et al., 2011, van de Wetering et al., 1998). Transmission can happen quickly; infections have been documented with only 5-10 minutes of probing/feeding (Wijkamp et al., 1996). Control of tomato spotted wilt (TSW) with insecticides that target the vector therefore relies upon disruption of thrips feeding behavior, either by killing the insect, causing it to leave the plant through antixenosis, or by altering its feeding behavior to make virus transmission less likely. Herein, we report on the effects of cyantraniliprole and imidaclorpid on feeding behavior of *F. fusca* and *F. occidentalis* on tomato through use of the electrical penetration graphing (EPG) technique. The EPG technique involves constructing a low-voltage circuit containing an insect and feeding substrate. The insect acts as a switch; when its mouthparts pierce the substrate to feed, the circuit is completed. While feeding, different feeding behaviors produce voltage fluctuations. The resultant voltage fluctuation waveforms are recorded on a computer. Various feeding behaviors such as probing
and ingestion have been associated with distinct waveforms, thereby allowing for an
assessment of insect activity during the recording (Walker, 2000).

2 MATERIALS AND METHODS

2.1 Thrips and plants

Adult *F. fusca* and *F. occidentalis* were obtained from laboratory colonies maintained
separately on *Phaseolus vulgaris* L. bean pods in controlled environments at 24 °C with ca. 60%
RH and a photoperiod of 14:10 LD (light:dark). Seeds of the thrips- and TSWV-susceptible
tomato cultivar ‘Florida 47’ were germinated in an insect-free greenhouse at 30 °C ± 3° under
natural light. Germination occurred in 72-cell plastic seed trays (Landmark Plastic Corporation,
Akron, OH, USA), containing potting media (Fafard, Agawam, MA, USA). Plants were maintained
for 21 days in these cells, at which point they had ca. 4 true leaves. Plants were then
transplanted into individual 300 mL plastic cups (Solo Cup Company, Lake Forest, IL, USA)
modified with a 25 mm diameter hole at the bottom for drainage, containing a soil mixture
consisting of 1:1:1 potting mixture (Fafard, Agawam, MA, USA), steam-sterilized loam, and
steam-sterilized sand.

2.2 Insecticide treatments

Immediately after transplant, plants received 50 mL of distilled water containing a
suspension of 20 mg a.i. per plant of cyantraniliprole (Cyazpyr™ 200 SC; DuPont, Delaware,
USA), or 29.7 mg a.i. per plant of imidacloprid (Admire® Pro; Bayer, Kansas City, MO, USA).
Untreated seedlings received 50 mL of distilled water with no insecticide. Following treatment,
plants were held until use in EPG experiments in an insect-free environment for 1 of 3 post-
treatment intervals: 2, 6, or 10 days after treatment (DAT). During this period plants received sufficient water to maintain moist soil without drainage through the bottom of the container.

2.3 EPG Setup

Adult thrips were selected from the colony 2-3 days after eclosion, and were affixed to a 2 cm long, 0.12 μm diameter gold wire (EPG Systems, Wageningen, The Netherlands) with a silver-based conductive glue made up of 1:1:1:1 silver flakes (KP-84, Ames Goldsmith Corporation, South Glens Falls, NY USA), distilled water, clear glue (Scotch Brand, St. Paul, MN, USA), and a 1000 ppm solution of Triton X-100 surfactant (Dow Chemical Company, Midland, MI, USA). Wires were fastened to thrips on the dorsal side of the mesothorax. Thrips were fasted for 30 minutes after being affixed to gold wires. Tomato plants were housed in Faraday cages to reduce electrical interference. EPG recordings were initiated when fasted thrips were placed on the youngest, fully expanded true leaf. Recordings lasted for 3 hours and were made using one Giga-4 DC-EPG system and one Giga-8 DC EPG system (EPG Systems, Wageningen, The Netherlands) with 1 GΩ of input resistance, allowing for a total of 12 simultaneous recordings. Separate experiments using a randomized design were conducted for *F. fusca* and *F. occidentalis*. For each species, separate experiments for each DAT interval were conducted as well. This allowed for 4 replicates of each insecticide treatment in each recording session, ultimately generating between 10 and 22 observations per species x treatment x DAT combination.

Stylet+ Software (EPG Systems, Wageningen, The Netherlands) was used to record and score waveforms generated by the EPG apparatuses. The EPG waveforms were manually scored to denote no probing, probing, and ingestion, which were based on previously-described
waveforms for *F. occidentalis* (Kindt et al. 2003, Kindt et al. 2006). Thrips that did not produce measureable electrical activity throughout the recording (i.e. became detached from the gold wire during the recording, fell from the plant, did not feed, etc.) were discarded from the dataset. The duration and number of occurrences of each waveform were compiled with a series of custom SAS programs based on the programs of Sarria et al. (2009). Programs were modified to more efficiently compile large amounts of probing data from multiple insects into a single dataset, and contained various error-checking algorithms to detect problems such as invalid waveforms and waveform sequences of the same type (e.g. “probing” followed by another “probing,” which should be punctuated by an “ingestion” or “no probing” event, and would denote an incorrectly scored waveform or missed scoring event). The resultant data were used to determine the number of probing events and number of ingestion events per insect. For insects that ingested during the recording (i.e. produced n ≥ 1 ingestion waveform), the total duration of ingestion (sum of all individual ingestion events), along with the mean duration of ingestion, were additionally calculated for each thrips.

All data were analyzed using PROC GLIMMIX in the SAS system, version 9.3 (SAS Institute, Cary, NC). The number of probing events was transformed to log(x). The number of ingestion events was transformed to log(x+1) so that all insects including those that probed but did not ingest during the recording period (i.e. number of probes ≥1, but number of ingestion events= 0), were included in this analysis. Further analyses of total ingestion duration and mean ingestion duration per ingestion event per thrips were conducted only on thrips that ingested (i.e. all values >0), therefore they were transformed to log(x). Per the experimental design, analyses for thrips species and days after treatment were conducted separately, and
included a main effect of insecticide treatment. The date of the recording was included as a random effect. Tukey's Honest Significant Difference (HSD) test was used post-hoc to conduct multiple comparisons on the least-squares means of each insecticide treatment at $\alpha=0.05$.

3 RESULTS

3.1 Insecticide effects on thrips probing

3.1.1 Frankliniella fusca. Insecticide treatment significantly affected the number of probes at 2, 6 and 10 DAT ($F_{2,48.33}=27.78$, $p<0.0001$; $F_{2,29.22}=38.45$, $p<0.0001$ and $F_{2,41.78}=75.40$, $p<0.0001$, respectively). At all DAT intervals, $F. fusca$ probed significantly fewer times on both cyantraniliprole- and imidacloprid-treated plants than on the water-treated plants. At each DAT interval, $F. fusca$ probed significantly less on imidacloprid-treated plants than on cyantraniliprole-treated plants (Figure 1).

3.1.2 Frankliniella occidentalis. Insecticide treatment significantly affected the number of probes at 2, 6, and 10 DAT ($F_{2,49}=10.66$, $p=0.0001$; $F_{2,49.89}=3.30$, $p=0.0449$; and $F_{2,53.98}=9.65$, $p=0.0003$, respectively). $Frankliniella occidentalis$ probed significantly fewer times on imidacloprid-treated than on water treated plants at 2, 6 and 10 DAT. However, the number of probes on cyantraniliprole-treated plants did not differ significantly from those on the water-treated plants at 2 DAT and was intermediate between the water- and imidacloprid-treated plants at 6 DAT. At 10 DAT, $F. occidentalis$ probed significantly fewer times on both cyantraniliprole- and imidacloprid-treated plants (Figure 1).

3.2 Insecticide effects on thrips ingestion

3.2.1 Frankliniella fusca. Significant effects of insecticide treatment on the number of ingestion events were seen at 2, 6, and 10 DAT ($F_{2,50}=4.50$, $p=0.016$, $F_{2,28.95}=11.21$, $p=0.0002$, $F_{2}$,
This analysis included *F. fusca* that probed during the recording, but did not ingest plant contents (n ingestion events = 0). At 2 DAT, the number of ingestion events on the water-treated plants was intermediate between the number of ingestion events on the cyantraniliprole- and imidacloprid- treated plants, with the number of events on cyantraniliprole-treated plants significantly higher than those on imidacloprid-treated plants. At 6 and 10 DAT the number of ingestion events per thrips was significantly greater on cyantraniliprole- than on water- or imidacloprid-treated plants (Figure 2).

Of the *F. fusca* that ingested, the total duration of ingestion was significantly affected by insecticide treatment at 2 and 6 DAT, but not at 10 DAT (F\(_{2,19.8}\) = 8.58, \(p=0.0021\), F\(_{2,12}\) = 7.16, \(p=0.0090\), F\(_{2, 20.4}\) = 2.14, \(p=0.1473\), respectively). At 2 and 6 DAT, the duration of ingestion was longer on cyantraniliprole-treated plants relative to water- and imidacloprid-treated plants (Figure 3).

Similarly, the mean duration of individual ingestion events was significantly affected by insecticide treatment at 2 and 6 DAT, but not at 10 DAT (F\(_{2,21.13}\) = 7.81, \(p=0.0029\), F\(_{2,12}\) = 4.28, \(p=0.0395\), F\(_{2,48.69}\) = 0.13, \(p=0.8816\). Post hoc means separations by the more conservative Tukey’s HSD however showed the mean duration of each ingestion event was longer on cyantraniliprole-treated plants relative to water- and imidacloprid-treated plants at 2 DAT only (Figure 4).

### 3.2.2 *Frankliniella occidentalis*

Significant effects of insecticide treatment on the number of ingestion events were observed at 2 DAT, but not at 6 or 10 DAT (F\(_{2,49}\) = 9.15, \(p=0.0004\), F\(_{2,49.03}\) = 1.83, \(p=0.1713\), F\(_{2,97.05}\) = 0.01, \(p=0.9901\), respectively). This analysis included individuals that probed during the recording, but did not ingest (n ingestion events= 0). At 2 DAT, the
number of ingestion events by *F. occidentalis* on cyantraniliprole-treated plants was greater than on water- or imidacloprid-treated plants (Figure 2).

Among the *F. occidentalis* that ingested, no effects of insecticide were seen on the total duration of ingestion at 2, 6, or 10 DAT ($F_{2,30.62}=1.88$, $p=0.1694$, $F_{2,24}=0.09$, $p=0.9159$, $F_{2,33.62}=0.09$, $p=0.9100$, respectively) (Figure 3), or on the mean duration of ingestion at 2, 6, or 10 DAT ($F_{2,27.66}=0.11$, $p=0.8953$, $F_{2,24}=0.47$, $p=0.6314$, $F_{2,35.59}=0.42$, $p=0.6589$, respectively) (Figure 4).

**4 DISCUSSION**

Both imidacloprid and cyantraniliprole have demonstrated efficacy in reducing the transmission and spread of TSW in previous studies. Greenhouse studies demonstrated that cyantraniliprole reduced TSW transmission in pepper against *F. fusca*, but failed to do so against *F. occidentalis* (Jacobson and Kennedy 2011). Imidacloprid has been demonstrated to suppress TSW transmission against *F. fusca* (Groves et al., 2001a). Against *F. occidentalis*, systemic applications of imidacloprid have demonstrated some activity in TSWV management in tomato, although it is often used in conjunction with foliar sprays of other insecticides to achieve desirable levels of suppression (Riley and Pappu 2004, Riley and Pappu 2000). While these previous studies demonstrated reductions in virus with these insecticides, the overall reported levels of TSW suppression has been variable (Chappell and Kennedy 2018).

EPG studies have determined that the number of probing events positively correlates with virus transmission, and the duration of ingestion during a probe negatively correlates with virus transmission, as sustained bouts of ingestion kill cells in the area of feeding, leaving the virus without viable tissue to inoculate (Kindt 2004, Stafford et al., 2011, van de Wetering et al.,
In our study, both cyantraniliprole and imidacloprid reduced the number of probes by both *F. fusca* and *F. occidentalis* but the magnitude of this effect was greater for *F. fusca*. The effects of these treatments on ingestion differed between species. For *F. fusca*, cyantraniliprole increased the number of ingestion events, the total duration of ingestion per thrips and the mean duration per ingestion event, with the magnitude of these effects tending to decrease with increasing time after treatment. For *F. occidentalis*, cyantraniliprole affected only the number of ingestion events at 2 but not 6 and 10 DAT and had no effect on the total duration of ingestion per thrips or the mean duration per ingestion event. In contrast, imidacloprid did not affect any of the ingestion parameters in *F. fusca* or *F. occidentalis* (Figure 2, Figure 3, Figure 4).

Similar results have been observed in previous EPG studies on pepper (*Capsicum annuum* L.), wherein cyantraniliprole and imidacloprid reduced probing in *F. fusca*, with cyantraniliprole-conferred reductions not consistently observed against *F. occidentalis* (Jacobson and Kennedy 2013). Previous EPG studies on tomato showed that imidacloprid reduced the frequency of probing by *F. fusca* (Joost and Riley 2005). Unlike our findings, Joost and Riley (2005) demonstrated that imidacloprid had an excitatory effect on *F. occidentalis*, with longer and more frequent probing behavior on imidacloprid-treated tomato, than on non-treated tomato. However, their tested rates were much lower than the one evaluated in this study.

Based on our results, both cyantraniliprole and imidacloprid would be expected to reduce virus transmission in tomato by modifying probing and ingestion behavior, albeit the mechanisms by which they do so are different. Imidacloprid reduces transmission by greatly reducing the number of probing events in both species. Cyantraniliprole may also reduce transmission by reducing probing by both species, but in *F. fusca*, there is an additional effect of
increased ingestion, which may also make transmission less likely. Our findings, along with previous EPG studies of insecticide effects on thrips feeding (Groves et al., 2001a, Joost and Riley 2005, Jacobson and Kennedy 2013) demonstrated that these insecticides act by reducing the probability that an individual viruliferous thrips probing on a treated plant will transmit TSWV. Because they do not entirely prevent the occurrence of probing events that are conducive to transmission, high infestation pressure resulting in large numbers of viruliferous thrips probing even briefly on treated plants can offset this reduced probability and result in inoculation and disease. Hence, the efficacy of these insecticides in TSW suppression is strongly influenced by the timing and abundance of viruliferous vectors that probe and feed on the treated crop (Chappell and Kennedy 2018).

Both *F. fusca* and *F. occidentalis* occur in many Southeastern USA agroecosystems. In these systems, *F. fusca* is often responsible for primary, early season spread of virus from overwintering weed hosts to young tomato plants, and *F. occidentalis* for secondary spread among tomato plants later in the season (Groves et al., 2001b, Groves et al., 2002, Joost and Riley 2004, Eckel et al., 1995). Thus, even though these insecticides, especially cyantraniliprole, tended to have a lesser effect on *F. occidentalis* feeding, suppression of primary spread of TSWV early in the season by *F. fusca* has the potential to reduce subsequent secondary spread within the crop field later in the season by, *F. occidentalis*. Imidacloprid is already widely used in such a manner, with at-plant/transplant applications used in tobacco, tomato, peanut, and pepper to suppress TSW transmission by *F. fusca* dispersing into crop fields from their winter annual weed hosts (Elbert et al., 2008, Groves et al., 2001a, Pappu et al., 2000, Riley and Pappu 2000, Riley and Pappu 2004, Riley 2007).
The suppressive effects these insecticides have in managing TSW involves more than their effects on feeding behaviors conducive to virus transmission. Previous studies have shown imidacloprid reduces settling by *F. fusca* on tomato (Joost and Riley 2005). Because thrips can only transmit TSWV if they have acquired it as a first larval instar (van de Wetering et al., 1996), secondary spread by *F. occidentalis* requires that thrips oviposit on, and the resulting larvae feed on, infected plants. A previous study has demonstrated that cyantraniliprole has a LC$_{50}$ that is 23 times lower in immature than adult *F. occidentalis*, and can sublethally impact adult fecundity and fertility (Bielza and Gullién 2014). While EPG studies are focused on observing insecticide effects on feeding behavior at the level of the individual insect, they serve as a valuable complement to greenhouse and field trials leading to a more complete understanding of insecticide efficacy in suppression of TSW and other insect vectored plant viruses.

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**Figure 1:** Number of probing events for *Frankliniella fusca* and *F. occidentalis* on water-, cyantraniliprole-, and imidacloprid-treated tomato plants at 2-, 6-, and 10- days after treatment (DAT). Bars with same letters within DAT interval do not significantly differ by Tukey’s HSD at α=0.05. Means separations are within species and DAT.
Figure 2: Number of ingestion events for *Frankliniella fusca* and *F. occidentalis* on water-, cyantraniliprole-, and imidacloprid-treated tomato plants at 2-, 6-, and 10- days after treatment (DAT). Bars with same letters within DAT interval do not significantly differ by Tukey’s HSD at $\alpha=0.05$. Means separations are within species and DAT.
**Figure 3:** Total duration of ingestion for *Frankliniella fusca* and *F. occidentalis* on water-, cyantraniliprole-, and imidacloprid-treated tomato plants at 2-, 6-, and 10- days after treatment (DAT). Bars with same letters within DAT interval do not significantly differ by Tukey’s HSD at \( \alpha=0.05 \). Means separations are within species and DAT. Only thrips that ingested at least one time during the EPG recording are presented here.
Figure 4: Mean duration of ingestion events for *Frankliniella fusca* and *F. occidentalis* on water-, cyantraniliprole-, and imidacloprid-treated tomato plants at 2-, 6-, and 10- days after treatment (DAT). Bars with same letters within DAT interval do not significantly differ by Tukey’s HSD at $\alpha=0.05$. Means separations are within species and DAT. Only thrips that ingested at least one time during the EPG recording are presented here.
CHAPTER 2: Temporal efficacy of neonicotinoid seed treatments against *Frankliniella fusca* on cotton

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**ABSTRACT**

**BACKGROUND:** Reports of neonicotinoid seed treatment (NST) failure against *Frankliniella fusca* in the Mid-South and Southeastern United States led to the discovery of widespread resistance in these insect populations. Previous studies of NSTs in other crops have shown the concentration of the insecticide to change over time, which could reduce its efficacy. To understand this relationship in cotton with *F. fusca*, our study examined how plant age alters the effects of NSTs (imidacloprid, imidacloprid+thiodicarb, thiamethoxam) by examining larval establishment at multiple seedling ages during the period of cotton seedling susceptibility to this insect. Additionally, we used *F. fusca* populations with differing neonicotinoid sensitivity levels to understand how resistance impacts this changing efficacy.

**RESULTS:** Greenhouse studies showed that larval numbers were significantly greater on older NST-grown cotton seedlings. The population with elevated neonicotinoid resistance had a more rapid increase in larval number on thiamethoxam-treated plants over time.

**CONCLUSION:** NSTs reduce the number of *F. fusca* larvae on younger seedlings, but this effect declines as seedlings age. The duration of efficacy is shorter against neonicotinoid-resistant populations. Neonicotinoid resistance in cotton-infesting *F. fusca* populations may be
accelerated by this time-dependent decrease in efficacy, which likely encourages low-dose exposure to these insecticides.

**KEYWORDS:** imidacloprid, thiamethoxam, tobacco thrips, insecticide resistance, *Gossypium hirsutum*
1 INTRODUCTION

Since their registration nearly two decades ago, neonicotinoid seed treatments (NSTs) have been one of the most important and effective tools for managing early season infestations of tobacco thrips (Frankliniella fusca Hinds) on cotton in the Mid-South and Southeastern United States. Adult *F. fusca* infest cotton seedlings early in the growing season, ovipositing into cotton leaves, and exposing the seedlings to damage from subsequent larval feeding. *Frankliniella fusca* larval feeding can lead to leaf damage, injure the meristem and disrupt apical dominance in the plant, reduce root development, and may reduce yield. Cotton seedlings are most vulnerable to *F. fusca* damage between emergence and the 4 to 5 true-leaf stage. In cotton, NSTs (imidacloprid and thiamethoxam) provide systemic insecticidal protection to cotton seedlings during this period of susceptibility to thrips damage. However, declining NST performance against *F. fusca* on seedling cotton in the Mid-South and Southeast United States has been reported, and resistance to both imidacloprid and thiamethoxam in *F. fusca* populations has been confirmed in both regions.

Resistance issues may be related to the seed treatment delivery method of neonicotinoids in cotton, which may increase the likelihood of low-dose selection. For NSTs, the effective concentration of insecticide present in a seedling decreases over time due to interacting factors such as plant growth, compound degradation, and metabolism. Temporal reduction in systemic neonicotinoid concentration has been observed in seed- and/or soil-applied neonicotinoids on multiple crops that utilize these technologies. Declining NST concentration over time in cotton may increase the possibility of *F. fusca* interacting with chronic, low concentrations of active ingredient, which may promote the selection for resistant
individuals in a population.\textsuperscript{21-23} NST-resistant \textit{F. fusca} have been shown to have higher oviposition and larval survivorship on NST treated plants than susceptible \textit{F. fusca}.\textsuperscript{24} Because NST concentration declines as the seedlings grow, this time by concentration interaction may favor selection for resistant insects and could be one explanation for the evolution of NST resistance in \textit{F. fusca} populations. The effects of declining neonicotinoid concentration on susceptible \textit{F. fusca} may provide insight into how NSTs select for resistant individuals. Understanding how this chronic exposure favors resistance selection is crucial for effective insecticide resistance management (IRM).

The overall objective of this study was to determine if \textit{F. fusca} larvae establish at higher levels as NST-grown cotton seedlings age. This information provides insight into a potential pathway for resistance selection on plants treated with systemic insecticidal seed dressings. Our second objective was to test if \textit{F. fusca} resistance status affects the rate of larval establishment as treated plants age. Because concentration of systemic neonicotinoids in cotton grown from treated seeds has been shown to decrease with plant age,\textsuperscript{20} we hypothesized that \textit{F. fusca} would establish more larvae on older plants. Further, we hypothesized that \textit{F. fusca} populations with elevated levels of neonicotinoid resistance would be more likely to oviposit into younger, NST-grown seedlings with putatively higher rates of systemic insecticide present, thereby more rapidly establishing populations of larvae.

2 METHODS

2.1 \textit{F. fusca} populations and their characterization

All field populations used in these experiments were collected from cotton producing areas in the United States Cotton Belt and their resistance levels were characterized (Table 1).
Populations were collected either by harvesting cotton seedlings early in the growing season from which adult *F. fusca* were collected, or using sweep nets to collect adult insects from the vegetation growing in cotton field margins at the beginning of the growing season. In a previous study, the type of host plant (i.e., field-grown cotton or non-crop weed species) had no detectible relationship with the measured resistance status of the population,\textsuperscript{15} indicating that collection from treated host plants would not significantly alter the population-level characterization of NST resistance measured in the laboratory. *Frankliniella fusca* populations were maintained on white cabbage (*Brassica oleracea* var. *capitata*) leaves in a controlled environment of 26°C with ca. 60% relative humidity and a photoperiod of 14:10 LD. The neonicotinoid-susceptible, North Carolina State University laboratory *F. fusca* colony (NCSU) was maintained in the laboratory under the same conditions as the field populations. Thiamethoxam and imidacloprid resistance was characterized in each population using the multiple-dose, diet-based bioassay approach described in Huseth et al.\textsuperscript{15} Briefly, 8 adult female *F. fusca* were placed in 1.5 mL microcentrifuge tubes (#05-408-130, Fisher Scientific, Pittsburgh, PA) capped with a sachet constructed using the excised cap of the tube and a 2 x 2 cm square of Parafilm (#PM992, Bemis Co., Neenah, WI USA). The sachets contained 155µL of a 3% sucrose + 0.05% green food dye (McCormick & Co. Inc., Hunt Valley, MD USA) solution that included a known concentration of formulated imidacloprid (Gaucho\textsuperscript{®} 600FS, 600 g imidacloprid L\textsuperscript{-1}, Bayer CropScience, Research Triangle Park, NC USA) or thiamethoxam (Cruiser\textsuperscript{®} 5FS, 600 g thiamethoxam L\textsuperscript{-1}, Syngenta Crop Protection, Greensboro, NC USA). Between six and nine concentrations (0-900 mg formulated insecticide L\textsuperscript{-1}) were used to estimate population-level susceptibility to neonicotinoids. Ten replicates were evaluated for each concentration. After
enclosure, insects were maintained for 48 hours in a temperature-controlled chamber at 27°C with 60%RH. At the end of the exposure period, the numbers of living and dead *F. fusca* were counted. Moribund insects unable to coordinate movement greater than one body length after stimulation with a fine brush were counted as dead. The bioassay data were analyzed to determine probit lines and LC50 values (see 2.3 Statistical Methods) (Table 1).

**2.2 Greenhouse experiments**

2.2.1 *Experimental design* Experiments were conducted at the Method Road Greenhouse Units on the campus of North Carolina State University in Raleigh, NC under a 16:8 LD photoperiod at 27±3°C. Cotton seeds were commercially treated with imidacloprid, imidacloprid+thiodicarb, or thiamethoxam (Table 2). All seeds received a seed-applied fungicide package of metalaxyl, penflufen, prothioconazole, and mycobutanil (Allegence®-FL, EverGol® Prime, Proline® 480SC, Bayer CropScience; Spera™ 240FS, Nufarm Agricultural Products, respectively). Cotton seedlings were grown to one of three ages before being infested by *F. fusca*: 9 days after planting (0-1 fully expanded true leaves), 14 days after planting (2-3 true leaves), and 19 days after planting (3-4 true leaves). Three separate experiments were conducted, each comparing the NCSU laboratory population to a field-collected thrips population from either Louisiana (LA) or Arkansas (AR). Two experiments, NCSU-vs-LA(1) and NCSU-vs-LA(2), utilized the cotton variety Stoneville 4946GLB2 and all three insecticide seed treatments (thiamethoxam, imidacloprid, imidacloprid+thiodicarb) alongside a fungicide-only control, allowing for 24 different thrips population x plant age x seed treatment combinations. The third experiment, NCSU-vs-AR, utilized the cotton variety Phytogen 333WRF, and the thiamethoxam and imidacloprid seed treatments alongside a fungicide only control, allowing for 18 different thrips population x plant
age x seed treatment combinations. A total of 360 cotton seedlings were used in each experiment, allowing for 15 replicates per level in the NCSU-vs-LA(1) and NCSU-vs-LA(2) experiments, and 20 replicates per level in the NCSU-vs-AR experiment. Both cotton varieties used (Stoneville 4946GLB2 and Phytogen 333WRF) are genetically engineered to express tolerance to herbicides and express insecticidal *Bacillus thuringiensis* proteins targeting Lepidoptera. These are not known to have an effect on *F. fusca*. A randomized complete block design was used in each experiment.

2.2.2. Growing conditions and sampling Cotton seeds were sown into individual 150mm-diameter clay pots at a depth of 25mm in a 2:2:1 sterilize soil mixture of peat-based potting mixture (2P potting mix; Fafard, Agawam, MA USA), loam, and sand, respectively. Each pot was covered with an individual insect-proof cage constructed from a 2-liter plastic beverage bottle (Pepsi Bottling Ventures, Garner, NC USA), which was modified by removing the bottom, removing the neck of the bottle to create a 35 mm diameter ventilation hole at the top of the cage, and cutting two additional 25 mm diameter lateral ventilation holes at the sides of the cage. All holes were covered with 150-µm screen (Midwest Filter Corporation, Lake Forest, IL USA) to provide air exchange. Approximately one-third of a 30 cm section of 3 mm-diameter drip irrigation tube (15FPEW53, Netafim USA, Fresno, CA USA) was threaded through an additional hole in each cage, and fixed in place with glue to allow for irrigation (Figure S1). Irrigation in the greenhouse was controlled by a timer to deliver ca. 63.5 mL of water per pot over a 3-minute interval every 6 h (total ca. 250 mL per day) to maintain adequate soil moisture without any drainage of water or insecticide through the bottom of the pot.
Planting dates were staggered so that seedlings of differing ages 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. Individual tubes were placed into each insect-proof cage and opened to release the thrips. *F. fusca* were allowed to infest for ten days before the seedlings were destructively sampled by clipping the hypocotyl. The 10-day infestation period was selected based on the temperature-dependent development time of *F. fusca*, and was adequate for eggs to hatch and the oldest larvae to develop through two larval instars at most without reaching the non-feeding pupal stage.

2.2.3 Quantifying larval establishment Sampled plants were placed into 260 mL polypropylene jars (#128070TSPP, Mold-Rite Plastics, Plattsburgh, NY USA) containing 150 mL of water and 250μL detergent. Immature thrips were separated from plant material by rinsing under running water through a series of two 20.3 cm diameter x 5.1 cm deep brass sieves (Dual Manufacturing Co., Inc., Franklin Park, IL USA). Coarse plant material was removed using a 500 micron (0.5 mm) mesh before the immature thrips were washed on a 150 micron (0.15 mm) mesh sieve with 70% EtOH into 20 mL disposable scintillation vials (#03-337-23, Fisher Scientific International Inc. Pittsburgh, PA). Extracted larvae were counted using a stereomicroscope.

2.3 Statistical analysis

To estimate the neonicotinoid resistant status of each population, insecticide LC50 values were calculated using PROC PROBIT in the SAS system version 9.3 (SAS Institute, Cary, NC). Surviving *F. fusca* at the end of the assay period were modeled as a binary outcome resultant of the natural log of insecticide concentration.
Larval counts were transformed to log(x+1) to meet the assumptions of normality and analyzed using PROC GLIMMIX in the SAS system version 9.3 (SAS Institute, Cary, NC). Plant age was considered a continuous variable. Preliminary analysis of control (no insecticide, fungicide only) plants in each experiment showed no effect of plant age on the number of larvae established, suggesting that varying host-plant quality related to changing plant age did not have an effect on larval establishment (Table S1). Because our goal was to directly test the period of activity for different NSTs on populations with varying neonicotinoid susceptibility, we chose to exclude the untreated control plants from further analysis. As a result, this analysis compares the performance of common NSTs used on the majority of commercial cotton grown in the Mid-South and Southeast. Analyses were sliced by active ingredient, with plant age and thrips population as main effects, along with their interaction.

3 RESULTS AND DISCUSSION

The NCSU population was evaluated alongside the Louisiana population in two separate trials, NCSU-vs-LA(1) and NCSU-vs-LA(2). For all three insecticide seed treatments evaluated across both trials, no significant effect of thrips population was observed (Table 3). Plant age was significant for all seed treatments aside from imidacloprid+thiodicarb in the NCSU-vs-LA(2) trial (Table 3). No significant thrips population x plant age interaction was observed for any seed treatment across both trials (Table 3). Given the similar resistance level of these two populations (Table 1), the lack of such population-related effects are not unexpected. These populations respond similarly to NSTs, with higher numbers of larvae established on older plants (Figure 1 and Figure 2), due to a putative decrease in effective insecticide concentration within the plant.
The NCSU population was evaluated alongside the Arkansas population in one trial, NCSU-vs-AR. A significant effect of thrips population was seen in the thiamethoxam evaluation, but not in the imidacloprid evaluation (Table 3). Plant age was significant for both seed treatments. No significant thrips population x plant age interaction was seen in either evaluation. The estimated LC$_{50}$ values for both thiamethoxam and imidacloprid are higher in the AR population compared to NCSU (Table 1). However, this difference is greater for thiamethoxam than imidacloprid (ca. 7.8X versus 5.5X, respectively), which may explain why a significant population effect was observed in the thiamethoxam evaluation but not the imidacloprid evaluation (Table 3). The lack of a significant thrips population by plant age interaction indicates that these populations, even with their differing resistance backgrounds, still exhibit a similar response to a change in effective insecticide rate, which was seen in our study as increased numbers of larvae established on older plants (Figure 3). Even with this similar response, the more rapid increase in larval number on older thiamethoxam-treated seedlings seen in the AR population relative to the NCSU population suggests that the duration of efficacy for an NST may be decreased when deployed against a population with an increased level of neonicotinoid resistance.

The results of our study highlight temporal decline in NST efficacy in cotton. While a decreasing efficacy may adequately control susceptible populations throughout the period of seedling susceptibility, the results of our NCSU-vs-AR evaluation suggest that a small increase in the level of resistance can increase larval establishment on younger plants. This, in turn, can cause thrips populations to more quickly reach economically injurious levels on susceptible seedlings. From an applied perspective, this change reduces the residual efficacy of NSTs
against F. fusca and necessitates additional insecticide sprays, both of which increase the cost of cotton production. This shortened duration of efficacy likely explains the commonly observed “failure” of NSTs in areas where cotton growers are contending with resistant F. fusca populations.

Another key result of this study is that F. fusca establish significantly more larvae on NST-treated plants as they age, which likely corresponds with a decrease in insecticide concentration in the plant. Here we clearly documented that even susceptible populations, such as NCSU and LA, can exploit this decrease in insecticide concentration and in turn establish more larvae on older, treated plants. Although this study did not directly document declining concentration within the plant, this pattern of decline has been previously reported for NSTs in cotton, and is expected to result in chronic exposure of larvae to low doses of insecticides. Across seasons, this cycle of chronic exposure to NSTs may favor resistant individuals in a population and drive long-term selection for resistant F. fusca populations. Similar trends have been observed in other crop systems, where exposure to variable pesticide concentrations in space and time are thought to affect the rate resistance development.

4 CONCLUSION

Our results show that the efficacy of NSTs against F. fusca decreases as cotton plants age. Frankliniella fusca can establish increased numbers of larvae on older treated plants, where they are exposed to a low dose of insecticide. Across seasons, this cycle of low-dose exposure may drive the development of resistance to NSTs. This trend was observed in F. fusca populations of different resistance backgrounds, with a more rapid increase in populations with measurably higher levels of neonicotinoid resistance. The more rapid increase reduces the
duration of efficacy of these seed treatments, which can be manifest as a reduced control or outright failure in the field. Neonicotinoid use in cotton has been widespread over time.² Additionally, these insecticides are utilized multiple times later in the growing season in cotton for control of other pests, allowing for prolonged exposure in *F. fusca* populations.³² As a result, traditional approaches to *F. fusca* IRM are confounded by the spatiotemporal extent of neonicotinoid use throughout the cotton production agroecosystem. This dependence on a narrow group of insecticides in several crops has likely driven the development of neonicotinoid resistance in *F. fusca* in the Mid-South and Southeast.¹⁶ As such, alternative management strategies such as insecticides with differing modes of action and shorter environmental persistence, cultural control practices such as conservation tillage and adjusted planting date to avoid peak *F. fusca* pressure,³³ and new plant-incorporated protectants with thrips activity³⁴ will likely be needed to control this pest moving forward.

**ACKNOWLEDGEMENTS**

We thank cooperating growers for providing access for insect collection on commercial farms. We thank Scott Martin, Kevin Langdon, and Shannon Morsello for assistance in collecting thrips populations. We thank Carol Berger, Marlee Franczak, and Daniel Grist for maintaining thrips colonies, Jesse Ditillo and Andrea Prestemon for assistance with sample processing, and Thomas Chappell with assistance with irrigation. We thank Bayer CropScience and Syngenta Crop Protection for supporting this research and providing treated cotton seed. This project was supported in part by the BASF Entomology Graduate Fellowship and Agriculture and Food
Research Initiative Competitive Grant no. 2015-70006-24281 from the USDA National Institute of Food and Agriculture.
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**Table 1** Resistance status of *F. fusca* populations utilized in the greenhouse evaluations

<table>
<thead>
<tr>
<th>Population</th>
<th>Origin</th>
<th>Experiment usage</th>
<th>Estimated LC$_{50}$ (ppm, ±95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Carolina State University (NCSU)</td>
<td>Laboratory</td>
<td>All</td>
<td>2.3 (1.6-3.0)</td>
</tr>
<tr>
<td>Louisiana (LA)</td>
<td>Field</td>
<td>NCSU-vs-LA(1)</td>
<td>7.3 (3.9-11.0)</td>
</tr>
<tr>
<td>Arkansas (AR)</td>
<td>Field</td>
<td>NCSU-vs-AR(1)</td>
<td>17.9 (13.4-23.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NCSU-vs-AR(2)</td>
<td>9.9 (5.7-14.6)</td>
</tr>
<tr>
<td>Active ingredient (AI)</td>
<td>Trade name</td>
<td>Manufacturer</td>
<td>Rate formulated insecticide ha(^{-1})</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------</td>
<td>------------------------------------</td>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>Cruiser 5FS</td>
<td>Syngenta Crop Protection, LLC, Greensboro, NC</td>
<td>0.09 L</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>Gaucho 600FS</td>
<td>Bayer CropScience LP, Research Triangle Park, NC</td>
<td>0.09 L</td>
</tr>
<tr>
<td>Imidacloprid + thiodicarb</td>
<td>Aeris</td>
<td>Bayer CropScience LP, Research Triangle Park, NC</td>
<td>0.18 L (0.375 mg imidacloprid + 0.375 mg thiodicarb)</td>
</tr>
</tbody>
</table>

\(^1\)liters formulated product per hectare was calculated using a stocking density of 143,518 seeds ha\(^{-1}\)
Table 3: Type III fixed effects of all experiments

<table>
<thead>
<tr>
<th>Seed Treatment</th>
<th>Parameter</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamethoxam</td>
<td>Population</td>
<td>3.98</td>
<td>0.0496*</td>
</tr>
<tr>
<td></td>
<td>Plant age</td>
<td>1.37</td>
<td>0.2456</td>
</tr>
<tr>
<td></td>
<td>Population x plant age</td>
<td>1.65</td>
<td>0.2020</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>Population</td>
<td>0.60</td>
<td>0.4424</td>
</tr>
<tr>
<td></td>
<td>Plant age</td>
<td>23.33</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td></td>
<td>Population x plant age</td>
<td>0.62</td>
<td>0.4330</td>
</tr>
<tr>
<td>Imidacloprid + thiodicarb</td>
<td>Population</td>
<td>0.28</td>
<td>0.5970</td>
</tr>
<tr>
<td></td>
<td>Plant age</td>
<td>11.23</td>
<td>0.0012***</td>
</tr>
<tr>
<td></td>
<td>Population x plant age</td>
<td>0.13</td>
<td>0.7193</td>
</tr>
</tbody>
</table>

Note: * indicates p-value < 0.05, ** indicates p-value < 0.01, *** indicates p-value < 0.001
Figure 1: *F. fusca* larvae recovered for both thrips populations in the NCSU-vs-LA(1) evaluation, along with the backtransformed model values, denoted by the solid and dotted lines.
Figure 2: *F. fusca* larvae recovered for both thrips populations in the NCSU-vs-LA(2) evaluation, along with the backtransformed model values, denoted by the solid and dotted lines.
Figure 3: *F. fusca* larvae recovered for both thrips populations in the NCSU-vs-AR evaluation, along with the backtransformed model values, denoted by the solid and dotted lines.
**SUPPLEMENTAL INFORMATION**

**Table S1** Effect of plant age on the number of established larval thrips on control seedlings (fungicide, no insecticide seed treatment) in all trials.

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>F-Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCSU-vs-LA(1)</td>
<td>1.25</td>
<td>0.2664</td>
</tr>
<tr>
<td>NCSU-vs-LA(2)</td>
<td>&lt;0.01</td>
<td>0.9926</td>
</tr>
<tr>
<td>NCSU-vs-AR</td>
<td>0.04</td>
<td>0.8481</td>
</tr>
</tbody>
</table>

**Table S2** Average thrips larvae per seedling (with standard error) for all *F. fusca* populations, plant ages and treatments tested.

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Population</th>
<th>19 days after planting</th>
<th>24 days after planting</th>
<th>29 days after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCSU-vs-LA(1)</td>
<td>Fungicide-only</td>
<td>NCSU</td>
<td>22.27 ± 4.41</td>
<td>18.71 ± 2.91</td>
<td>22.07 ± 3.43</td>
</tr>
<tr>
<td></td>
<td>(control)</td>
<td>LA</td>
<td>33.00 ± 4.38</td>
<td>27.82 ± 5.48</td>
<td>19.14 ± 3.46</td>
</tr>
<tr>
<td></td>
<td>Thiamethoxam</td>
<td>NCSU</td>
<td>12.20 ± 3.40</td>
<td>18.46 ± 3.17</td>
<td>12.93 ± 3.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA</td>
<td>8.08 ± 1.73</td>
<td>22.14 ± 3.59</td>
<td>17.80 ± 2.51</td>
</tr>
<tr>
<td></td>
<td>Imidacloprid</td>
<td>NCSU</td>
<td>1.38 ± 0.40</td>
<td>8.57 ± 2.46</td>
<td>9.93 ± 2.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA</td>
<td>2.87 ± 0.58</td>
<td>4.20 ± 1.14</td>
<td>10.40 ± 2.53</td>
</tr>
<tr>
<td></td>
<td>Imidacloprid +</td>
<td>NCSU</td>
<td>2.13 ± 0.88</td>
<td>3.93 ± 1.08</td>
<td>7.79 ± 2.40</td>
</tr>
<tr>
<td></td>
<td>thiodicarb</td>
<td>LA</td>
<td>4.08 ± 1.65</td>
<td>6.14 ± 2.38</td>
<td>8.64 ± 2.38</td>
</tr>
<tr>
<td>NCSU-vs-LA(2)</td>
<td>Fungicide-only</td>
<td>NCSU</td>
<td>27.79 ± 4.80</td>
<td>25.57 ± 3.69</td>
<td>24.23 ± 3.39</td>
</tr>
<tr>
<td></td>
<td>(control)</td>
<td>LA</td>
<td>32.08 ± 4.60</td>
<td>34.57 ± 3.24</td>
<td>31.69 ± 4.90</td>
</tr>
<tr>
<td></td>
<td>Thiamethoxam</td>
<td>NCSU</td>
<td>16.54 ± 4.04</td>
<td>21.50 ± 4.13</td>
<td>25.27 ± 3.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA</td>
<td>15.20 ± 3.09</td>
<td>25.71 ± 3.79</td>
<td>26.86 ± 4.60</td>
</tr>
<tr>
<td></td>
<td>Imidacloprid</td>
<td>NCSU</td>
<td>3.40 ± 1.28</td>
<td>3.38 ± 1.00</td>
<td>8.50 ± 2.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LA</td>
<td>1.62 ± 0.54</td>
<td>5.33 ± 1.09</td>
<td>9.27 ± 2.11</td>
</tr>
<tr>
<td></td>
<td>Imidacloprid +</td>
<td>NCSU</td>
<td>5.33 ± 2.16</td>
<td>5.36 ± 1.63</td>
<td>5.13 ± 1.27</td>
</tr>
<tr>
<td></td>
<td>thiodicarb</td>
<td>LA</td>
<td>3.87 ± 1.64</td>
<td>5.53 ± 1.59</td>
<td>4.29 ± 1.11</td>
</tr>
<tr>
<td>NCSU-vs-AR</td>
<td>Fungicide-only</td>
<td>NCSU</td>
<td>31.37 ± 4.42</td>
<td>15.85 ± 1.74</td>
<td>36.95 ± 5.74</td>
</tr>
<tr>
<td></td>
<td>(control)</td>
<td>AR</td>
<td>63.10 ± 6.31</td>
<td>41.90 ± 5.48</td>
<td>58.89 ± 5.04</td>
</tr>
<tr>
<td></td>
<td>Thiamethoxam</td>
<td>NCSU</td>
<td>2.11 ± 0.65</td>
<td>7.70 ± 1.72</td>
<td>10.35 ± 1.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AR</td>
<td>15.45 ± 1.61</td>
<td>31.10 ± 3.97</td>
<td>48.60 ± 6.28</td>
</tr>
<tr>
<td></td>
<td>Imidacloprid</td>
<td>NCSU</td>
<td>5.00 ± 1.37</td>
<td>5.80 ± 1.46</td>
<td>8.32 ± 1.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AR</td>
<td>5.75 ± 1.68</td>
<td>36.85 ± 4.95</td>
<td>15.15 ± 2.86</td>
</tr>
</tbody>
</table>
Figure S1 a: Overview of cage design showing (1) top ventilation hole, (2) emitter threaded into cage, and (3) lateral ventilation holes. b: A cotton seedling growing inside of a cage c: Multiple cages deployed in the greenhouse off of a central irrigation line.
CHAPTER 3: Determining *Frankliniella fusca* egg distribution in neonicotinoid seed treated cotton

ABSTRACT

**INTRODUCTION:** *Frankliniella fusca* 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).

**METHODS:** 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.

**RESULTS:** 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.
1 INTRODUCTION

Neonicotinoid-resistant tobacco thrips, *Frankliniella fusca* (Hinds), present an increasingly serious obstacle to affordable and sustainable cotton production in the southeastern USA (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 (Huseth et al., 2016, Elbert et al., 2008). Although NSTs effectively controlled these insects for nearly 20 years, 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.

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 are thigmotactic (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.
2 METHODS

2.1 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). 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; Spera™ 240FS, Nufarm Agricultural Products, respectively) for pathogen management. NST seeds additionally received an insecticidal active ingredient (thiamethoxam or imidacloprid) at the labeled rate of 0.375mg ai seed$^{-1}$.

2.2 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% relative humidity and a photoperiod of 16:8 LD. For field studies, the resident *F. fusca* population was allowed to naturally infest seedlings. To determine the neonicotinoid resistance levels of these insects, 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.

### 2.3 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\(^{\circ}\)C under a 16:8 LD light cycle. Cotton seeds were sown individually at a depth of 25mm into 150mm-diameter terra cotta pots containing a soil mixture of 2:2:1 potting mixture (Fafard, Agawam, MA, USA), 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 L beverage bottle, and received water through an automated system delivering ca. 63.5mL of water per pot over a 3-minute interval every 6 h (total ca. 250mL/day), 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 (0-1 true leaves), 14 days after planting (2-3 true leaves), and 19 days after planting (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, Reisig 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
1.5mL 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 days, allowing enough time for oviposition but not egg hatching (Lowry et al., 2014, Watts 1934) before the seedlings were destructively sampled by clipping the hypocotyl.

2.4 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.

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 260mL polypropylene jars (#128070TSPP, Mold-Rite Plastics, Plattsburgh, NY USA) containing 150mL of water and 250µL detergent.

2.5 Seedling processing

Clipped greenhouse and field seedlings were returned to the laboratory where they were cleared and stained with a previously described lacto-phenol, acid fuschin 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 four minutes 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 L⁻¹ acid fuchsin solution for one minute to stain leaves. Stained plant material was spread
onto 150mm diameter plastic Petri dishes (Cat No: 25384-094, VWR, Radnor, PA USA), 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, USA) to create
digital image files. The files were analyzed using the GNU Image Manipulation Program version
2.8 (The GIMP Team) 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 Supplemental
Information.

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, USA). 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 20mL vials (#03-337-23, Fisher Scientific International Inc., Pittsburgh, PA, USA), and counted with a stereomicroscope.

2.6 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.

3 RESULTS

3.1 F. fusca resistance levels

The laboratory F. fusca population had LC50 values of 2.2ppm (CL 1.8ppm-3.0ppm) for thiamethoxam and 0.9ppm (CL 0.8ppm-1.2ppm) for imidacloprid. The field F. fusca population had LC50 values of 204.4ppm (89.9ppm-664.3ppm) for thiamethoxam and 30.9ppm (CL 18.2ppm-64.2ppm) for imidacloprid in 2016, and 41.0ppm (CL 22.8ppm-94.7ppm) for thiamethoxam and 26.5ppm (CL 15.9ppm-53.8ppm) for imidacloprid in 2017.

3.2 Greenhouse experiment

3.2.1 Egg counts Significant effects of insecticide treatment, and a significant insecticide treatment x leaf type were seen at all plant ages. A significant effect of leaf type was seen at 9- and 14-days after planting, but not at 19-days after planting (Table 1). The greenhouse F. fusca population oviposited significantly more eggs into the fungicide-only treated seedlings at 9- and
14-days after planting (41.9 eggs, standard error (SE) 32.8-31.1, 30.4 eggs, SE 23.2-37.6, respectively) relative to the thiamethoxam (12.7 eggs, SE 9.0-16.5, 9.5 eggs, SE 6.9-12.1, respectively) and imidacloprid treatments (2.2 eggs, SE 1.7-2.7, 3.6 eggs, SE 2.3-4.8, respectively), and more eggs relative to the imidacloprid treatment at 19-days after planting (19.6 eggs, SE 15.9-23.2 versus 4.7 eggs, SE 3.7-5.7). 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-days after planting (Figure 1).

3.2.2 Egg density Analyses of density showed significant effects of insecticide treatment, and a significant insecticide treatment x leaf type at all plant ages. A significant effect of leaf type was seen at 9- and 14-days after planting, but not at 19-days after planting (Table 1). Density patterns were similar to those observed with egg numbers (Figure 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 (Figure 2), which was not observed in the egg number analysis (Figure 1).

3.3 Field experiments

3.2.1 Egg counts Significant effects of leaf type and trial year were seen at both sample dates. Neither a significant insecticide treatment x 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 (Figure 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 (Figure 3).

3.2.2 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 x 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 (Figure 4).

3.2.3 Larval counts Significant effects of insecticide treatment, leaf type, and trial year were seen in both samples. No significant insecticide treatment x 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 (Figure 5).

4 DISCUSSION

4.1 Greenhouse findings

As seen in previous studies (Huseth et al., 2017), NSTs reduced oviposition relative to untreated plants, which given the resistance background of the laboratory-raised *F. fusca* population is expected (Figure 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-days after planting, egg numbers are uniform between cotyledons and true leaves in the imidacloprid- and thiamethoxam-treated seedlings. In the fungicide-only plants, significantly
more eggs are still found in the cotyledons. At 9 days after planting however, significantly more
eggs are found in cotyledons relative to true leaves in all treatments (Figure 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. Neonicotinoid seed
treatments altered this preference however, with more equal, albeit lower, densities of eggs
seen in both tissue types (Figure 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 (Figure 2). Aging cotyledons becoming less
preferable for oviposition could also cause some of the oviposition shift to true leaves in older
plants, but 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.

4.2 Field findings- oviposition

Unlike patterns observed in the greenhouse, insecticide treatment did not reduce egg numbers in the field (Figure 3). This is reflective of the resistant background of the field population, which had LC$_{50}$ values that were at least 18.6- and 29.4x-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 (Figure 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 (Figure 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 (Figure 3, Figure 4). Together these results show that resistant thrips do not display strong behavioral avoidance to these compounds and instead utilize the preferred cotyledon tissue for oviposition on insecticide-
treated plants. Egg numbers and density alike to did not significantly differ on the cotyledons versus true leaves in the imidacloprid- and thiamethoxam-treated plants at the second sample date (Figure 3, Figure 4). This is similar to patterns observed in the greenhouse on older, treated plants (Figure 1, Figure 2). However, mean numbers of thrips eggs were higher on cotyledons than true leaves in the field population (Figure 1), but were higher on true leaves than cotyledons in the greenhouse population (Figure 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 LC50 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.

4.3 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 towards 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 (Figure 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 (Figure 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*.

### 4.4 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. 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 (Reisig 2015, Collins et al., 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 are able to 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), are registered for use on cotton, and have demonstrated activity against *F. fusca*, including NST-resistant populations (D’Ambrosio et al., 2018b, Huseth et al., 2017). 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.

**ACKNOWLEDGEMENTS**

We thank Marlee Franczak, Andrea Prestemon, Carol Berger, Sean LaFata, and Jesse Ditillo for assistance with sample procurement and processing and thrips rearing. We thank Dan Mott and Dominic Reisig for assisting with field trial logistics. We thank Thomas Chappell for assistance with automated irrigation setup. We thank Bayer CropScience for providing treated cotton seed and funding this project. This project was also funded by the BASF Entomology Graduate Fellowship.
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### Table 1: Type III effects for egg number and egg density in greenhouse experiments with a neonicotinoid-susceptible *F. fusca* population

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Plant age</th>
<th>Parameter</th>
<th>Num. df</th>
<th>Denom. df</th>
<th>F-value</th>
<th>Pr&gt;F</th>
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<td>Egg number</td>
<td>9 days after planting (0-1 true leaves)</td>
<td>Insecticide treatment (IT)</td>
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<td>38</td>
<td>24.65</td>
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<td>14 days after planting (2-3 true leaves)</td>
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<td>IT x LT</td>
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* p<0.05, **p<0.01, ***p<0.001
Table 2: Type III effects for egg number, egg density, density and larval distribution in field experiment involving a neonicotinoid-resistant *F. fusca* population

<table>
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<th>Distribution</th>
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<th>Parameter</th>
<th>Num. df</th>
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<th>F-value</th>
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<td>19.35</td>
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* p<0.05, **p<0.01, ***p<0.001
Figure 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 days after planting interval do not significantly differ. Means separations by Tukey’s HSD at $\alpha=0.05$. 
Figure 2: *Frankliniella fusca* egg density with standard error in the greenhouse study involving a neonicotinoid-susceptible laboratory population. Bars with the same letters within days after planting interval do not significantly differ. Means separations by Tukey’s HSD at α=0.05.
Figure 3: *Frankliniella fusca* egg 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 $\alpha=0.05$. 
Figure 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 α=0.05.
**Figure 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 $\alpha=0.05$. 
**SUPPLEMENTAL INFORMATION**

**Table S1:** Mean egg numbers per seedling by plant part (± SE) for both oviposition experiments. Percent of eggs oviposited in each plant part are denoted in parentheses.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sample (seedling development)</th>
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<th>Thiamethoxam</th>
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<td></td>
<td>Cotyledon</td>
<td>True leaf</td>
<td>Cotyledon</td>
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<tr>
<td><strong>Greenhouse</strong></td>
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<td></td>
<td></td>
<td></td>
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<td>1 (0-1 true leaves)</td>
<td>66.9± 6.2 (99.7%)</td>
<td>0.2± 0.2 (0.03%)</td>
<td>17.5± 4.2 (98.3%)</td>
<td>0.3± 0.3 (1.7%)</td>
</tr>
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<td>2 (2-3 true leaves)</td>
<td>56.0± 8.6 (92.1%)</td>
<td>4.8± 1.6 (7.9%)</td>
<td>11.9± 4.6 (62.6%)</td>
<td>7.1± 2.4 (37.4%)</td>
</tr>
<tr>
<td>3 (3-4 true leaves)</td>
<td>29.3± 5.4 (74.9%)</td>
<td>9.8± 2.2 (25.1%)</td>
<td>10.2± 3.6 (35.1%)</td>
<td>18.9± 3.8 (64.9%)</td>
</tr>
<tr>
<td><strong>Field</strong></td>
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<td></td>
</tr>
<tr>
<td>1 (ca. 2 true leaves)</td>
<td>32.7± 4.6 (98.5%)</td>
<td>0.5± 0.3 (1.5%)</td>
<td>23.1± 3.6 (95.9%)</td>
<td>1.0± 0.5 (4.1%)</td>
</tr>
<tr>
<td>2 (ca. 4 true leaves)</td>
<td>14.3± 4.5 (81.3%)</td>
<td>3.3± 0.6 (18.8%)</td>
<td>7.6± 1.4 (73.1%)</td>
<td>2.8± 0.7 (26.9%)</td>
</tr>
</tbody>
</table>
Table S2: Mean leaf area (cm$^2$) per seedling by plant part (± SE) for both oviposition experiments. Percent area constituted by each plant part is denoted in parentheses.

<table>
<thead>
<tr>
<th>Experiment (seedling development)</th>
<th>Sample</th>
<th>Untreated</th>
<th>Thiamethoxam</th>
<th>Imidacloprid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenhouse experiment (susceptible F. fusca)</td>
<td>1 (0-1 true leaves)</td>
<td>24.5± 1.0 (89.7%)</td>
<td>23.7± 1.0 (84.9%)</td>
<td>23.8± 1.2 (78.0%)</td>
</tr>
<tr>
<td></td>
<td>2 (2-3 true leaves)</td>
<td>24.3± 1.4 (62.6%)</td>
<td>27.1± 1.1 (64.5%)</td>
<td>24.6± 1.0 (64.4%)</td>
</tr>
<tr>
<td></td>
<td>3 (3-4 true leaves)</td>
<td>26.6± 1.8 (56.1%)</td>
<td>26.5± 2.2 (59.8%)</td>
<td>26.1± 1.7 (59.2%)</td>
</tr>
<tr>
<td>Field oviposition experiment (resistant F. fusca)</td>
<td>1 (ca. 2 true leaves)</td>
<td>13.9± 0.7 (64.7%)</td>
<td>14.6± 1.1 (56.8%)</td>
<td>16.5± 1.2 (49.4%)</td>
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<tr>
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<td>2 (ca. 4 true leaves)</td>
<td>11.1± 0.8 (30.2%)</td>
<td>16.2± 1.4 (40.3%)</td>
<td>15.0± 1.2 (24.9%)</td>
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</tbody>
</table>
Figure S1: Cotton seedling area by plant part with standard error in the greenhouse study involving a neonicotinoid-susceptible *F. fusca* population. Bars with similar letters within sample do not significantly differ. Means separations by Tukey’s HSD at $\alpha=0.05$. 
Figure S2: Cotton seedling area by plant part with standard error in the field study involving a neonicotinoid-resistant *F. fusca* population. Bars with similar letters within sample do not significantly differ. Means separations by Tukey’s HSD at α=0.05.
CHAPTER 4: Electrical penetration graphing analysis of *Frankliniella fusca* feeding behavior on neonicotinoid seed treated-cotton: effects of insecticide resistance and plant age

ABSTRACT

**INTRODUCTION:** *Frankliniella fusca* resistance to neonicotinoid seed treatments (NSTs) is an increasingly difficult obstacle to cotton production in the Southeast and Mid-South United States. NSTs were previously discovered to have a nonlethal suppression effect on oviposition of neonicotinoid-susceptible *F. fusca* but not on neonicotinoid-resistant *F. fusca*. Understanding the pre-oviposition feeding interaction between the adult *F. fusca* and NST-grown cotton seedlings could both provide more insight on this effect, and inform future management strategies of this pest.

**METHODS:** Electrical penetration graphing (EPG) was used to record the probing and ingestion of *F. fusca* adults from neonicotinoid-resistant and susceptible populations on NST (thiamethoxam and imidacloprid)-grown cotton seedlings. Recordings were conducted on plants of varying ages to document any time-related decreases in residual NST efficacy.

**RESULTS:** Both NSTs reduced the number of probing events by the susceptible thrips with the magnitude of this reduced on older seedlings. Resistant *F. fusca* feeding behaviors were not affected by NSTs regardless of seedling age. NSTs did not alter ingestion in either population. These results showed that NSTs alter feeding by reducing the number of probing and ingestion events, not reducing ingestion duration, and suggest a link between probing frequency and oviposition likelihood.
1 INTRODUCTION

For nearly 20 years, neonicotinoid seed treatments (NSTs) have been used to successfully manage *Frankliniella fusca* (Hinds) in cotton. However, recent reports of NST field failures lead to surveys that documented the occurrence of neonicotinoid-resistant *F. fusca* populations throughout the US Cotton Belt (Huseth et al., 2016). Better understanding of the biology of *F. fusca* in cotton may offer insight into how *F. fusca* is ultimately exposed to and interacts with the NST, how the interaction with an NST differs between susceptible and resistant insects, and what measures may be taken to manage resistance with this insect moving forward.

On cotton, *F. fusca* injury is a result of adult insects ovipositing onto seedlings and the resultant larvae feeding on developing plant tissue (Cook et al., 2011). Previous studies of *F. fusca* biology on cotton showed that neonicotinoid seed treatments have a nonlethal, suppressive effect on adult *F. fusca* oviposition (Huseth et al., 2017). This effect is diminished against resistant insects (Huseth et al., 2017), allowing them to establish higher numbers of larvae more rapidly on NST-grown plants (D’Ambrosio et al., 2018a). Previous studies have suggested a correlation with thrips feeding frequency and oviposition frequency by the closely related species *F. occidentalis* (Pergande) (Whittaker et al., 2004). As such, the nonlethal effect on oviposition may result from the adults encountering the systemic insecticide while initially feeding on the cotton seedling, then departing from the plant in response before ovipositing. However, this pre-oviposition feeding aspect of *F. fusca* biology in cotton is not well understood. Previous EPG studies have shown imidacloprid to reduce *F. fusca* probing on wild mustard, pepper, and tomato (Groves et al., 2001, Joost and Riley 2005, Jacobson and Kennedy
These probing reductions have been linked with reduced settling behavior (Joost and Riley 2005) and reduced larval numbers (Groves et al., 2001). Determining if NSTs induce similar effects on probing and feeding by adult *F. fusca* on cotton could offer a more complete understanding of the NST’s suppressive effect on oviposition. Further, determining whether or not NSTs reduce probing and feeding by resistant insects will increase the understanding of how these insects overcome NSTs in the field. This knowledge can be used to offer improved insecticide resistant management (IRM) strategies in the future.

Herein, we use electrical penetration graphing (EPG) to examine the pre-oviposition adult feeding responses of neonicotinoid-resistant and –susceptible *F. fusca* with NST treated plants. EPG has been used to study the feeding behavior of a wide variety of sucking insects, including thrips (Kindt et al., 2003, Kindt et al., 2006, Jacobson and Kennedy 2013 Groves et al., 2001, Joost and Riley 2005, Stafford et al., 2011). The technique involves constructing an electrical circuit that includes both the insect and plant. Once the insect pierces the plant to feed, the circuit is complete, and resultant voltage waveforms can be recorded on a computer. Distinct voltage waveforms have been associated with specific feeding behaviors in *Frankliniella* species thrips in previous studies (Figure 1) (Kindt et al., 2003, Kindt et al., 2006, Stafford et al., 2011). We examined *F. fusca* feeding behavior on cotton seedlings treated with the two most common NSTs: imidacloprid and thiamethoxam. We compared the feeding behaviors of a laboratory-raised, susceptible *F. fusca* population with that of a neonicotinoid-resistant field *F. fusca* population. Finally, based on previous studies showing NST efficacy against *F. fusca* decreases over time as plants become larger (D’Ambrosio et al., 2018a), we examined three separate plant ages to see if feeding behavior on plants grown from neonicotinoid treated
seeds changed over the 19 day period following planting. The selected ages encompassed the period of vulnerability to thrips injury in cotton, which is reported to be between emergence and the production of 4-5 true leaves (Cook et al., 2011, Reisig 2014, Reisig 2016). We hypothesized that resistant \( F. \textit{fusca} \) would feed more readily on NST-grown seedlings than susceptible thrips, and that age-related decreases in NST efficacy would allow \( F. \textit{fusca} \), regardless of their resistance background, to feed more on older seedlings.

2 MATERIALS AND METHODS

2.1 Thrips and resistance status characterization

The neonicotinoid-susceptible, North Carolina State University laboratory \( F. \textit{fusca} \) colony (NCSU) was maintained on white cabbage (\textit{Brassica oleracea}, var. “capitata”) in a controlled environment of 24°C with ca. 60% RH under a 14:10 LD photoperiod.

Neonicotinoid-resistant \( F. \textit{fusca} \) were obtained from a commercial cotton production region in Northampton County, NC (36.4119 N, -77.5322 W). Insects were collected with sweep nets on 2 and 9 May 2017 by sweeping vegetation in field margins and winter wheat growing in fields that were planted with NST-grown cotton in the previous year. Adult female \( F. \textit{fusca} \) were separated from the sweep sample by-catch (e.g., plant parts and other arthropods) with an aspirator, and placed into culture under the same conditions as the NCSU colony. Field collected adults (\( F_0 \)) were allowed to reproduce on cabbage until their progeny reached maturity. Adult \( F_1 \) \( F. \textit{fusca} \) were subsequently used in EPG experiments. Unused \( F_1 \) individuals were kept in culture under the same conditions, and allowed to produce \( F_2 \) offspring.

To characterize their resistance backgrounds, both the NCSU population and the \( F_2 \) field population generation were subject to a previously described, multiple-dose diet bioassay.
(Huseth et al., 2016) to determine 50% lethal concentrations ($LC_{50}$) for both imidacloprid and thiamethoxam. Dose response data were analyzed using logistic regression with PROC GLIMMIX in the SAS System, version 9.3 (SAS Institute, Cary, NC). Surviving thrips were modeled as a binary outcome of the log(x+1) of insecticide dosage. This produced a dose coefficient (slope) and intercept for each active ingredient in both populations. These terms were used to inversely calculate $LC_{50}$ estimates (Huseth et al., 2016). Confidence intervals were calculated by using the 95% confidence intervals of the dose coefficient.

2.2 Cotton seedlings

All cotton seeds (cv Stoneville 4946GLB2) were commercially treated with a mixture of metalaxyl, penfluifen, prothioconazole, and mycobutanil (Allegence®-FL, EverGol® Prime, Proline® 480SC, Bayer CropScience; Spera™ 240FS, Nufarm Agricultural Products, respectively) to protect against pathogens. In addition, NST seeds received an insecticidal active ingredient of thiamethoxam (Cruiser 5FS, Syngenta Crop Protection, LLC, Greensboro, NC) or imidacloprid (Gaucho 600F, Bayer CropScience, Research Triangle Park, NC) at the labeled rate of 0.375mg a.i seed$^{-1}$. Stoneville 4946GLB2 expresses Bacillus thuringiensis traits targeting chewing insect pests of cotton, but these traits are not known to have activity against $F. fusca$.

Cotton seeds were sown individually into 150mm-diameter terra cotta pots at a depth of 25mm. Pots were filled with 2:2:1 potting mixture (Fafard, Agawam, MA, USA), steam-sterilized loam, and steam-sterilized sand. Pots were held in a greenhouse at 30°C± 5°C under ambient light. Each pot was individually irrigated by an automated irrigation system calibrated to deliver ca. 65mL of water to each plant daily, allowing for adequate soil moisture without leaching of water or insecticide through the bottom of the pot.
2.3 EPG Setup and waveform processing

Adult thrips selected from each colony 2-3 days after their emergence were affixed to a 2 cm long, 0.12 µm diameter gold wire (EPG Systems, Wageningen, The Netherlands) with a silver-based conductive glue made up of 1:1:1:1 silver flakes (KP-84, Ames Goldsmith Corporation, South Glens Falls, NY, USA), distilled water, clear glue (Cat. 6050, Scotch Brand, St. Paul, MN, USA), and a 1000 ppm solution of Triton X-100 surfactant (Dow Chemical Company, Midland, MI, USA). Wires were glued to the insect at the dorsal side of the mesothorax. Thrips were fasted for 30 minutes after being affixed to gold wires. Cotton seedlings were carefully uprooted and the roots and basal portion of the stem placed into 75 mL, 3 cm-diameter glass jars filled with distilled water and containing an EPG electrode. The tap root of each seedling was then cut underwater with a pair of sharp scissors to prevent cavitation in the xylem and maintain plant turgor. EPG recordings were initiated once fasted thrips were placed on the abaxial surface of one of the cotyledons. Recordings lasted for 2 hours and were made using one Giga-4 DC-EPG system and one Giga-8 DC EPG system (EPG Systems, Wageningen, The Netherlands) with 1 GΩ of input resistance, allowing for a total of 12 simultaneous recordings. A randomized design was implemented. Recordings were conducted separately for thrips population and plant age, allowing for 4 replicates for each of the 3 insecticide treatments during each recording session. Although each recording session only contained a single plant age, plant ages were randomly distributed across recording sessions throughout the entire experimental period.

EPG waveforms were scored using Stylet+ software, version 1.3 (EPG Systems, Wageningen, The Netherlands). Waveforms were scored as probing, not probing, or ingesting
Waveforms were compiled with a series of programs in the SAS System, version 9.3 (SAS Institute, Cary, NC), based on procedures used by Backus et al. (2007) and Sarria et al. (2009). The resultant dataset contained the number of occurrences of each waveform and their duration for each individual insect recorded by the EPG apparatus. The number of probing events, number of ingestion events, total duration of ingestion events, and mean duration of ingestion events were used for analysis.

2.4 Statistical analysis

Data were log transformed, and analyzed using PROC GLIMMIX in the SAS System, version 9.3 (SAS Institute, Cary, NC). The model included insecticide treatment and plant age as fixed effects, along with their interaction. Plant age was treated as a continuous variable. Recording date was included as a random effect. Dunnett’s test was used post hoc to compare the two insecticide treatments to the untreated seedlings. Analyses were conducted separately by thrips population.

3 RESULTS AND DISCUSSION

3.1 Thrips resistance background

The NCSU laboratory population had LC$_{50}$ values of 2.2ppm (1.8-3.0 95% confidence limits (CL)) for thiamethoxam, and 0.9ppm (CL 0.8-1.2) for imidacloprid. The F$_2$ field population had LC$_{50}$ values of 30.2ppm (CL 13.9-113.7) for thiamethoxam, and 11.7ppm (CL 6.5-30.2) for imidaclorpid. The larger confidence intervals around the field population estimates suggested some heterogeneity for resistance (i.e. individuals have varying levels of resistance within the population). This was similarly observed by Huseth et al. (2016).
3.2 Effects of insecticide on number of probes

The neonicotinoid-susceptible thrips from the laboratory population probed significantly fewer times on the thiamethoxam- and imidacloprid-treated seedlings than on the fungicide-only controls (Figure 2). The main effect of plant age on the number of probes was not significant but the plant age x insecticide interaction was significant (Table 1). These results indicate that both imidacloprid and thiamethoxam seed treatments reduce probing in neonicotinoid susceptible *F. fusca*. As treated seedlings grow older, the number of probes per thrips increased on NST-grown seedlings (Figure 3), reflecting a decrease in residual efficacy of these seed treatments as the seedlings grow. In a previous study, reductions in larval mortality and oviposition suppression by these NSTs with increasing seedling age have been documented (D’Ambrosio et al., 2018a).

A different trend was seen in the neonicotinoid-resistant field population. Neither the main effects of plant age or insecticide treatment, nor the plant age x insecticide treatment interaction effect on the number of probing events were significant (Table 1). This absence of effect reflected the resistance of this population to both thiamethoxam and imidacloprid, and demonstrated that resistant *F. fusca* were not deterred by NSTs when probing cotton seedlings (Figure 2), even when seedlings were young and the concentration of systemic insecticide was expected to be highest (Zhang et al., 2011)(Figure 3).

3.3 Effects of insecticide on thrips ingestion

Similar to the effects on the number of probes, the neonicotinoid-susceptible thrips ingested significantly fewer times on the thiamethoxam- and imidacloprid-treated seedlings than on the fungicide-only controls (Figure 4). Plant age also significantly affected the number
of ingestion events, with thrips ingesting more on older plants (34.3 ingestion events at 9 DAP across all treatments, standard error (SE) 24.3-44.3, versus 49.5 ingestion events at 19 DAP, SE 39.8-59.3). The insecticide treatment x plant age interaction was not significant (Table 1). Plant age, insecticide treatment, and their interaction did not affect the number of ingestion events in the field resistant population (Table 1).

For neonicotinoid-susceptible thrips, neither insecticide treatment nor the plant age x insecticide treatment interaction significantly affected the total duration of ingestion (Table 1). However, plant age significantly affected the total duration, with thrips ingesting longer on older plants (635.8 seconds at 9 DAP across all treatments, SE 508.1-763.5, versus 1622.8 seconds at 19 DAP, SE 1439.2-1806.4). For thrips from the neonicotinoid-resistant field population, the effects of plant age, insecticide treatment, and the plant age x insecticide treatment interaction on total duration of ingestion were not significant in (Table 1). When analyzing the mean duration of each ingestion event, no effects of plant age, insecticide, treatment, or their interaction, were seen in either population (Table 1).

Examining the effects of NSTs on both F. fusca probing and ingestion provides a more complete understanding of the F. fusca-NST interaction. Sustained bouts of ingestion kill localized areas of cells and are responsible for characteristic thrips injury (van de Wetering et al., 1998). Additionally, the effects of NSTs on ingestion can show when F. fusca detects the insecticidal compound: before probing without mouthpart contact, when the mouthparts are engaged in the plant and in contact superficially with insecticidal compound before ingesting, or once the compound has entered the alimentary tract after ingestion begins. The reductions in the number of ingestion events on treated plants but lack of effects on total and mean
ingestion duration indicated that NSTs primarily affect *F. fusca* feeding either by preventing or interrupting the probing event early on, before the insect began to ingest plant contents. This suggests the insect can detect the insecticide before contacting the plant with its mouthparts, or while its mouthparts are in contact with the plant, without ingesting the compound into its alimentary tract (Figure 1).

### 3.4 Implications of feeding on oviposition, future management

These results show that neonicotinoid susceptible *F. fusca* probe and ingest fewer times on cotton seedlings grown from neonicotinoid treated seed. This effect is lost in neonicotinoid-resistant populations, which correlates with previously-described antioviposition effects of NSTs against susceptible *F. fusca*, which is similarly lost in resistant *F. fusca* (Huseth et al., 2017). As NST-grown seedlings become larger and the effective concentration of systemic insecticide decreases (Zhang et al., 2011), susceptible *F. fusca* probe more readily. This is consistent with previous studies of *F. fusca* reproduction on cotton, in which *F. fusca* will more readily establish larvae on older NST-grown seedlings (D’Ambrosio et al., 2018a). Altogether, these results suggest a link between *F. fusca* probing and oviposition, in which *F. fusca* will probe the plant before ovipositing to determine its quality as an oviposition substrate. Higher-quality substrates (i.e., plants with low/no systemic insecticide present) are probed and have their contents ingested more often, and are subsequently more often oviposited into. NST-grown plants are probed, the insecticide is detected, and oviposition does not occur. Resistant *F. fusca* do not perceive the systemic insecticide as a deterrent, and probe, ingest, then ultimately oviposit normally.
Systemic insecticides with differing modes of action that are perceived by *F. fusca* before ingestion like the NSTs may also provide nonlethal oviposition suppression. This both reduces ultimate plant injury by limiting larval recruitment, and reduces selection pressure, as the insecticide-susceptible individuals are not killed and removed from the population. This low selection pressure may explain in part why NSTs performed well against this insect for many years without regular rotation to other modes of action. Having new chemistries, with multiple modes of action and a similar suite of effects could provide similar, long-term, satisfactory management of *F. fusca* in cotton. Previous studies (Huseth et al., 2017, D’Ambrosio et al., 2018a) have characterized many details of the interaction between *F. fusca* and cotton seedlings grown from imidacloprid or thiamethoxam treated seeds. With this study, we now understand there are similar antifeedant effects that follow the same pattern as the antioviposition effect. Having a comparable understanding of future strategies employed against *F. fusca* in cotton, including alternative insecticides (Huseth et al., 2017, D’Ambrosio et al., 2018b), different cotton varieties (Wann et al., 2017), and transgenic traits (Bachman et al., 2017), could facilitate development of effective and sustainable management strategies targeting this pest.

**ACKNOWLEDGEMENTS**

We thank Marlee Franczak and Andrea Prestemon for assistance with thrips collection, EPG scoring, and greenhouse maintenance, Sean LaFata and Jesse Ditillo for assistance with thrips collection and greenhouse maintenance, Thomas Chappell for assistance with EPG analysis, and Carol Berger for assistance with thrips colony maintenance. Cotton seed was
provided by Bayer CropScience. This project was funded by the BASF Entomology Graduate Fellowship and Bayer CropScience.
REFERENCES


### Table 1: Type III fixed effects for all analyzed EPG parameters in both F. fusca populations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population</th>
<th>Effect</th>
<th>Num d.f.</th>
<th>Denom d.f.</th>
<th>F-value</th>
<th>Pr&gt;F</th>
</tr>
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<tbody>
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<td>Number of probing events</td>
<td>Laboratory susceptible</td>
<td>Plant age</td>
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<td>28.86</td>
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<td>Insecticide treatment</td>
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<td>147.5</td>
<td>5.58</td>
<td>0.0046**</td>
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<td>Plant age x Insecticide treatment</td>
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<td>145.4</td>
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<td>Laboratory susceptible</td>
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<td>21.99</td>
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*-p<0.05, **-p<0.01, ***-p<0.001
Figure 1: Typical *F. fusca* EPG waveform on cotton showing electrical baseline with no mouthpart engagement in plant, scored as “not probing” (a), beginning of probing event, when mouthparts are inserted into the plant, scored as “probing” (b), movement of mouthparts within plant and salivation without ingestion, also scored as “probing” (c), and ingestion of plant contents, scored as “ingesting” (d). Based on the findings of this study, insecticide detection likely occurs before mouthpart contact or during (c). This figure depicts 43 seconds of recording.
Figure 2: Mean number of probing events across all plant ages for both *F. fusca* populations. Insecticide treatment bars (gray shaded) followed by an asterisk (*) significantly differ from the fungicide-only reference (white) within thrips population by Dunnett’s test at $\alpha=0.05$. 
Figure 3: Mean number of probing events by plant age for both *F. fusca* populations (±SE).
Figure 4: Mean number of ingestion events across all plant ages for both *F. fusca* populations. Insecticide treatment bars (gray shaded) followed by an asterisk (*) significantly differ from the fungicide-only reference (white) within thrips population by Dunnett’s test at $\alpha=0.05$. 
CHAPTER 5: Evaluation of alternative mode of action insecticides in managing neonicotinoid-resistant *Frankliniella fusca* in cotton

Chapter published in *Crop Protection*


**ABSTRACT**

**BACKGROUND:** *Frankliniella fusca* (Hinds) resistance to neonicotinoid seed treatments (NSTs) used in cotton has created a need for more diverse insecticide options targeting thrips. Alternative insecticides must protect seedlings while they are most vulnerable to *F. fusca* injury (emergence through five true leaves). In this study, we evaluated non-neonicotinoid foliar insecticide sprays currently registered for use on cotton against a neonicotinoid resistant *F. fusca* population.

**METHODS:** During two-seasons, we compared NSTs (imidacloprid, imidacloprid + thiodicarb, and thiamethoxam) to non-neonicotinoid foliar sprays of acephate, spinetoram, abamectin, cyantraniliprole, and cyantraniliprole + abamectin in field trials to evaluate their efficacy against a neonicotinoid resistant *F. fusca* population. Applications were made to both early- and full-maturity cotton varieties (Stoneville 4946GLB2 & 6448GLB2) to examine *F. fusca* larval establishment, plant vigor, and seed cotton yield.

**RESULTS:** With the exception of abamectin, foliar insecticide treatments consistently reduced *F. fusca* larval numbers and minimized true leaf damage at a level equal to or greater than NSTs. Yield was not affected by insecticide treatment. Non-neonicotinoid foliar sprays have utility in managing neonicotinoid-resistant *F. fusca* and should be recommended to alleviate selection
pressure against NSTs in cotton and unnecessary economic losses due to ineffective NST use against resistant *F. fusca* populations.

**KEYWORDS:** spinetoram, cyantraniliprole, abamectin, acephate, resistance management, tobacco thrips
Cotton producers in the Southeast and Mid-South United States manage a complex of insect pests throughout the growing season each year. Of these, the tobacco thrips, \textit{Frankliniella fusca} (Hinds) is the most important early season insect pest of cotton (Cook et al., 2011). Adult \textit{F. fusca} infest and oviposit into newly emerged cotton seedlings early in the growing season. The resultant larvae hatch and feed on the seedlings, injuring leaves (Cook et al., 2011), inhibiting root development (Sadras and Wilson, 1998), and can disrupt apical dominance (Gaines 1934). In severe infestations, seedlings may die from \textit{F. fusca} damage or be more vulnerable to environmental stress (Cook et al., 2011), at times resulting in reduced yield (Bauer and Roof 2002, Rummel and Quisenberry 1979, Watts 1937).

Historically, carbamates and organophosphate insecticides were applied in-furrow at planting, along with foliar sprays of the same modes of action to control \textit{F. fusca} (Cook et al., 2011). The registration of user-friendly neonicotinoid insecticide seed treatments (NSTs, Insecticide Resistance Action Committee mode of action [IRAC MoA] group 4A), coupled with loss of registration of older insecticides, led to an overreliance on NST for thrips control across the US Cotton Belt (Cook et al., 2011, Elbert et al., 2008). For more than a decade, NSTs effectively protected cotton seedlings through the period of \textit{F. fusca} susceptibility, which has been widely established to be from seedling emergence until 4-5 true leaves (Bacheler and Mott, 2004, Cook et al., 2011, Fromme and Batchelor, 2002, Herbert and Malone, 2004, Hopkins et al., 2002, Johnson et al., 2003, Reisig 2016, Reisig, 2014). However, in recent years, reports of reduced NST control led to the discovery of widespread resistance to imidacloprid and thiamethoxam NSTs in \textit{F. fusca} populations throughout the Southeast and Mid-South
(Huseth et al., 2016). Currently, many growers use supplemental foliar acephate sprays to control resistant thrips on NST cotton (Brown 2017, Lorenz 2013, Reisig 2018, Stewart 2016,).

Responses to reduced NST efficacy due to resistance have included supplemental neonicotinoid in-furrow applications in addition to NSTs (Hart 2014, Stewart 2016, Stewart 2014), increases in foliar sprays (Stewart 2014), and a resurgence of aldicarb soil treatment use (Attaway 2016, Lorenz 2016, Stewart 2016). While these supplemental insecticides may provide relief to growers, diversifying thrips management options will be important to mitigate resistance long term. Insecticides with different modes of action than neonicotinoids and activity against thrips, such as spinetoram (IRAC MoA group 5), cyantraniliprole (IRAC MoA group 28) and abamectin (IRAC MoA group 6), are currently registered for *F. fusca* control on cotton and could be incorporated into an insecticide resistance management (IRM) program designed to alleviate selection pressure for resistance to neonicotinoids. These alternative insecticides have lower mammalian toxicity and pesticide applicator safety risks than organophosphates and carbamates (Dripps et al., 2008, Grosso et al., 2012, Sattelle et al., 2008), and would increase the number of MoAs used for *F. fusca* to mitigate the evolution of resistance to individual *F. fusca* insecticides.

In this field study, we examined alternative MoA insecticides applied as foliar sprays to control a neonicotinoid resistant *F. fusca* population. We hypothesized that non-neonicotinoid insecticides would more effectively control neonicotinoid resistant thrips than NSTs. To document the response of neonicotinoid resistant thrips to alternative MoAs, we measured the density of immature *F. fusca* larvae that established on treated cotton. We also tested two cotton varieties to document the response of *F. fusca* to varieties with different seedling vigor.
This assessment was designed to document the relationship between insecticide use and duration in the thrips susceptibility window (i.e., more rapid seedling growth reduces the required time for insecticide protection). To do this, we quantified multiple measures of seedling vigor and associated these responses to insecticide treatments using multiple regression. Results of this study compare the performance of non-neonicotinoid MoAs to NSTs against neonicotinoid resistant *F. fusca* under field conditions. We show that these alternative MoAs can have superior performance when compared to standard NSTs against neonicotinoid resistant thrips populations and could form a foundation for *F. fusca* IRM programs in the US Cotton Belt.

2 METHODS

2.1 Trial location, *F. fusca* population, and seeds

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 spacing at a seeding rate of 14 seeds m⁻¹, for a stocking density of 143,518 seeds ha⁻¹. Trials were planted in a randomized complete block design with four replications. Blocks were separated with 1.5 m alleys of bare soil. Planting took place in early May of each year (Table 1). Planting date was adjusted to maximize the likelihood of high *F. fusca* pressure using the North Carolina Climate Office Thrips Infestation Predictor for Cotton (TIP) (https://climate.ncsu.edu/cottonTIP). The TIP tool was released to the public on 1 April 2017. As such, the public release version of this tool was used to inform the 2017 planting date. We used a closed beta release of TIP in 2016.
Two cotton varieties, Stoneville 4946GLB2 and Stoneville 6448GLB2 (Bayer CropScience, Research Triangle Park, NC, USA), were selected for these trials. Both varieties contained herbicide tolerance traits for glyphosate and glufosinate-ammonium, along with insecticidal proteins *Bacillus thuringiensis* Cry1Ac and Cry2Ab2 targeting lepidopteran pests (neither of which are known to have activity against *F. fusca*). These two varieties differ in their maturity period, with Stoneville 4946GLB2 having an earlier maturity than Stoneville 6448GLB2 (hereafter called ST4946 and ST6448 respectively). They were selected based on their regional suitability for the field location (https://www.cropscience.bayer.us/products/seeds/stoneville-cotton/variety-overview). Maturity differences may relate in part to increased seed size, as larger seeds have been shown to have higher vigor potential than smaller seeds (Snider et al., 2014). A preliminary analysis of a randomly selected subset of seeds from each variety confirmed that ST4946 seeds were on average heavier (n=200 seeds, $F_{1, 198}=473.1$, $p<0.001$), longer (n=100 seeds, $F_{1, 98}=90.62$, $p<0.001$), and wider (n=100 seeds, $F_{1, 98}=45.99$, $p<0.001$) than ST6448 seeds. Aside from thrips control, extension recommended practices for North Carolina were used (Edmisten et al., 2018).

In both years, the infesting *F. fusca* population was evaluated for neonicotinoid resistance. Four plots of non-NST cotton randomly distributed within our field of treatments were allowed to grow without insecticide application until the second sample date, when they were destructively harvested by cutting stems at soil level and placing seedlings into 20 L buckets in single-seedling deep layers separated with a triple layer of paper towels for transport to the laboratory. Upon return to the laboratory, seedlings were placed into 4.9 L plastic tubs (VP-173302, PFS Sales Company, Raleigh, NC, USA), modified with 100 mm-diameter holes on
the base and lid (VP-1257064, PFS Sales Company, Raleigh, NC, USA) covered in 150-µm screen (Midwest Filter Corporation, Lake Forest, IL USA) to promote airflow for drying. Tubs were provisioned with leaves of white cabbage (Brassica oleracea L. var. capitata) on which the larval thrips were reared to adult. Emerged adult thrips were initially identified to species visually, as the more uniform, dark color of female F. fusca is distinct from other cotton-infesting thrips species in North Carolina, which are either noticeably lighter in color (e.g. other Frankliniella spp., Thrips tabaci) or distinctly striped (e.g. Neohydatothrips variabilis). A random subset of the visually-identified, putative F. fusca was slide mounted and morphologically identified to species using a compound light microscope (Palmer et al., 1992). Morphological identification confirmed these individuals were F. fusca. While the exact proportion of F. fusca in these samples was not determined due to the large number of insects produced, we visually estimated that >95% were F. fusca. Adult female F. fusca that developed on this cabbage were subjected to a diet-based multiple dose assay to calculate the 50% lethal concentration (LC50) values to both imidacloprid and thiamethoxam (D’Ambrosio et al., 2018, Huseth et al., 2017, Huseth et al., 2016). The North Carolina State University NST-susceptible laboratory population of F. fusca was used as a reference for the LC50 levels calculated for the field-collected F. fusca population. LC50 calculations were based on the methodology of Huseth et al., and involved modeling the binary outcome of insect survivorship in the bioassay with logistic regression as a function of the log(x+1) dose using PROC GLIMMIX in the SAS System, Version 9.3 (SAS Institute, Cary, NC) (2016). For each population, this produced estimates of a dose coefficient (slope), along with an intercept. Inversely predicted confidence intervals were calculated by using the confidence intervals of the dose coefficient estimate.
2.2 Insecticide application

All cotton seeds were treated with a base application of metalaxyl, penflufen, prothioconazole, and mycobutanil (Allegence®-FL, EverGo® Prime, Proline® 480SC, Bayer CropScience; Spera™ 240FS, Nufarm Agricultural Products, Alsip, IL, USA respectively) to manage seedling pathogens. NSTs included an insecticidal active ingredient (thiamethoxam, imidacloprid, or imidacloprid + thiodicarb) in addition to the base fungicide application at the labeled field rate (Table 2). Foliar sprays were applied with a CO₂ powered backpack sprayer connected to a spray boom consisting of two flat fan spray nozzles (TR8002VS, TeeJet Technologies, Wheaton, IL, USA) spaced 0.9 meters apart calibrated to deliver foliar sprays at a rate of 93.54 L ha⁻¹. Nozzles were centered over the seedlings 30 cm above the soil surface during application. Each foliar insecticide was delivered at the highest labeled rate for cotton (Table 2). With the exception of acephate sprays, a 0.5% v/v nonionic surfactant (Induce®, Helena Chemical, Collierville TN) was added spray mixes to improve spray distribution and coverage on cotton seedlings. All applications were made once the first true leaf began to emerge on >80% of cotton seedlings (Table 1).

2.3 Data collection

2.3.1 Larval sampling Eight cotton seedlings were randomly selected from the leftmost two rows (4 per row) of each four row plot, and placed into 260 mL polypropylene jars (#128070TSPP, Mold-Rite Plastics, Plattsburgh, NY USA) containing 150 mL of water and 250 µL detergent. Plant samples were rinsed under running water through two brass sieves, 20.3 cm diameter by 5.1 cm deep (Dual Manufacturing Co., Inc., Franklin Park, IL, USA). Samples were first passed through a 500 micron mesh sieve to remove larger pieces of plant material and
debris while allowing thrips larvae to pass through, then through a 150 micron mesh sieve to collect thrips larvae (Rummel and Arnold, 1989). Larval thrips were rinsed from the final sieve with 70% EtOH into 20 mL scintillation vials (#03-337-23, Fisher Scientific International Inc., Pittsburgh, PA, USA). Collected larvae were counted with a stereomicroscope.

2.3.2 Plant vigor metrics Five seedlings were randomly selected from the leftmost two rows of each four row plot. Seedlings were cut flush with the soil surface with a pair of scissors, placed in a plastic zipper storage bag, and held in a cooler for transport to the laboratory. Each plant was then measured with a ruler from the cut base to the growth point to determine above-ground height. The number of expanded true leaves (width ~10 mm or greater) on each plant was counted, and plants were weighed to obtain seedling wet weight. Three of the five plants were placed into paper bags and held in a growth chamber at 40°C with 15% RH for five days, at which point they were weighed again to obtain the dry weight of above ground growth.

2.3.3 Frankliniella fusca injury ratings Injury was assessed by walking the rows in each plot and observing signs of thrips injury in the rightmost two rows of each plot. Using an industry standard rating index (Herbert 1996), F. fusca injury was scored between 0 (no injury) and 5 (seedling death). Severity was determined by the amount of feeding injury observed on the plant along with whether or not emerging true leaves were normally-sized or reduced/injured due to thrips feeding.

2.3.4 Seed cotton yield Five to seven days following the last thrips sample, a stand count was taken from the rightmost two rows of each plot to determine the total plant population for both rows (Table 1). At the conclusion of the growing season, plants were chemically defoliated with a mixture of S,S,S-tributyl phosphorotrithioate, thidiazuron, and ethephon (Folex® 6 EC,
Amvac Chemical Corporation, Los Angeles, CA, USA; Dropp® 50WP, Prep®, Bayer CropScience) and lint was harvested with a two row cotton picker (John Deere Model 9930, Deere and Co., Moline, IL USA) (Table 1). A per plant seed cotton yield was determined by dividing the total yield per plot by the number of plants.

2.3.5 Statistical analysis A correlation analysis was conducted to associate the four measured plant vigor metrics (height, wet weight, dry weight, and true leaf counts) using the “corrplot” package (Wei and Simko, 2017) in R, version 3.4.4 (R-Core team). This analysis showed that vigor metrics were significantly and positively correlated with one another (Table 3). Because of these correlations, we chose true leaf count as a single representative vigor metric for subsequent analyses. Larval counts, expanded true leaf count, injury ratings, and seed cotton yield were transformed to log(x+1) to meet assumptions of normality. Data were analyzed using PROC GLIMMIX in the SAS System Version 9.3 (SAS Institute, Cary, NC). Analyses included main effects for insecticide treatment, cotton variety, and the days after planting that a sample was obtained (DAP), along with all possible two-way interactions and their three-way interaction. Because we wanted to understand the general performance of insecticide treatments between years, trial year was included as an additional main effect to account for inter-annual variation in thrips infestation pressure and environmental conditions. Experimental block was included as a random effect. To account for the repeated observations of plots within the trials, an additional random effect of DAP was included, in which the repeatedly observed subject, “plot,” was specified. A main effect of days after planting and associated interactions were not included in seed cotton yield analyses, as this metric is only obtained once each year. Because NSTs have been shown to decrease in efficacy against *F. fusca* as cotton seedlings grow older.
(D’Ambrosio et al., 2018), we treated DAP as a continuous variable to assess any time-related change in insecticide efficacy. Because the purpose of this trial was to compare the efficacy of the alternative foliar insecticides alongside existing NSTs, we excluded the untreated controls from this analysis to directly compare insecticide performance. Thrips density and injury rating summary statistics for all treatments, including the untreated, are provided in the Supplemental Material.

A final analysis was conducted on larval numbers at the first sample date to determine how the resistance status of the field *F. fusca population would affect insecticide performance relative to the untreated control seedlings when the effective concentrations of all insecticides were putatively highest. Data were analyzed using PROC GLM in the SAS System, Version 9.3 (SAS Institute, Cary, NC), and included the main effects of insecticide treatment, cotton variety, and trial year. The insecticide treatment x cotton variety interaction was initially included in the model, but was found to not be significant and was subsequently removed. Dunnett’s test was used post hoc to compare the performance of each insecticide treatment in reducing larval numbers to the untreated control seedlings.

3 RESULTS AND DISCUSSION

3.1 *Frankliniella fusca* resistance status

Resistance bioassays of the field *F. fusca population estimated an LC$_{50}$ of 204.4ppm (95% confidence limits (CL) of 89.9-664.3 ppm, reduced chi-square ($\chi^2$) = 2.37) and 30.9 ppm (18.2-64.2 ppm CL, $\chi^2 = 2.93$) for thiamethoxam and imidacloprid respectively in 2016, and 41.0 ppm (22.8-94.7 ppm CL, $\chi^2 = 1.81$) and 26.5 ppm (15.9 ppm-53.8 ppm CL, $\chi^2 = 2.07$) for thiamethoxam and imidacloprid respectively in 2017. The North Carolina State University
laboratory susceptible population had estimated LC$_{50}$ values of 2.2 ppm (1.8-3.0 ppm CL, $\chi^2_v = 1.74$) and 0.9 ppm (0.8-1.2 ppm CL, $\chi^2_v = 1.73$) for thiamethoxam and imidacloprid respectively. Overall, this shows that the field $F$. fusca were ca. 17.8-88.9x resistant to thiamethoxam, and ca. 29.4-34.3x resistant to imidacloprid relative to the neonicotinoid susceptible reference population.

3.2 Larval counts

Significant main effects of insecticide treatment, cotton variety, and DAP were observed. We also documented a significant insecticide treatment x DAP interaction (Table 4). Overall, with the exception of abamectin, seedlings treated with foliar sprays had fewer $F$. fusca larvae throughout the study period relative to NST-treated seedlings (Figure 1). Because this $F$. fusca population was resistant to neonicotinoid insecticides, the relative difference between NSTs and alternative MoA insecticides suggests that rotation away from neonicotinoids could be a significant benefit for growers. Among foliar-applied insecticides, cyantraniliprole and spinetoram performed similarly to acephate, the industry standard. Minimal abamectin efficacy, coupled with the high efficacy of both cyantraniliprole and the cyantraniliprole + abamectin treatments, suggests that cyantraniliprole is the primary insecticidal component in reducing thrips larval numbers in the dual active ingredient treatment. Similar results about the performance of abamectin against neonicotinoid resistant $F$. fusca have been shown in previous laboratory efficacy studies (Huseth et al., 2017).

On average, more larvae were recovered from the larger, more vigorous ST4946 seedlings (34.8 thrips/8 seedlings, standard error (SE) 32.0-37.6) than the ST6448 seedlings (27.1 thrips/8 seedlings, SE 25.1-29.1). Differences in seedling size in a randomized complete
block design (RCBD) experimental setting may drive this unequal infestation between varieties, wherein *F. fusca* may more readily infest the larger ST4946 plants (See 3.3). However, the insecticide treatment x cotton variety interaction was not significant, indicating that the effect of insecticide performance did not depend on cotton variety.

Previous studies indicate that NSTs have a combination of an antioviposition effect on adult *F. fusca* coupled with some direct lethal activity against larvae that end up on these plants. The strength of these effects was reduced against neonicotinoid-resistant *F. fusca* populations (Huseth et al., 2017). Here, larval numbers on NST-grown seedlings suggest that resistance-related behavior may affect population dynamics in the field, with the resident neonicotinoid resistant *F. fusca* ultimately establishing more larvae on NST-treated cotton compared to cotton sprayed with alternative MoAs. This result suggests that these foliar applied MoAs are viable NST alternatives for *F. fusca* control in areas where neonicotinoid resistance is an issue.

### 3.3 True leaf count and injury

For true leaf count, significant effects of insecticide treatment, cotton variety, and DAP, along with a significant insecticide treatment x DAP interaction were observed (Table 4).

On average, thiamethoxam-treated seedlings had the fewest true leaves, suggesting that the resident, thiamethoxam-resistant *F. fusca* were able to injure these plants by impeding normal leaf production. True leaf count is one measure of seedling health that can be linked to insecticide performance against thrips injury (Cook et al., 2011, Reisig 2016, Reisig 2014). Our data show that the foliar sprays had an equivalent or greater number of true leaves than NSTs (Figure 2). Researchers also use visual rating indices when assessing NST thrips activity.
(Copeland et al., 2016, Knight et al., 2015, Taylor et al., 2017) and economic value (North et al. 2018). Visible injury ratings were not significantly different among insecticide treatments when we controlled for effects of year, variety, DAP, and experimental block in our overall model ($F_{7,236.3} = 0.82, p = 0.5735$).

The larger-seeded ST4946 had more true leaves than the smaller-seeded ST6448 on average (4.8 true leaves during trial period, SE 4.7-4.9 versus 3.8 true leaves, SE 3.7-3.9 respectively). Larger seedlings may be more attractive to ovipositing *F. fusca* in a RCBD study, and may explain the occurrence of significantly more larvae on ST4946 than ST6448 seedlings (See 3.2). However, no significant insecticide treatment x cotton variety interaction was observed for true leaf count, indicating that the effect of cotton variety was independent of insecticide performance.

### 3.4 Seed cotton yield

A significant effect of year was observed on seed cotton yield, but the effects of insecticide treatment, cotton variety, and any interactions were not significant (Table 4). This result suggests that all treatments perform equally in terms of reducing *F. fusca* injury and protecting yield potential. However, an analysis of the data set including the untreated controls also revealed the absence of significant effects of insecticide treatment ($F_{8,119} = 0.96, p = 0.4688$), cotton variety ($F_{1,119} = 0.87, p = 0.3528$) and their interaction ($F_{8,119} = 1.28, p = 0.2623$). This result is not necessarily unexpected. Thrips-related impacts on yield are indirect, and may vary significantly under different environmental conditions (Cook et al., 2011). Our trials likely had favorable environmental conditions following the thrips susceptibility period, allowing for injured seedlings to recover and/or yield compensation. An analysis of stand count supports
this, and showed that even with untreated seedlings included, insecticide treatment had no effect on the ultimate post-sample period stand count ($F_{8,122} = 0.40, p=0.9161$).

3.5 Implications of F. fusca resistance status on insecticide evaluation

In this study, the largest difference in insecticide efficacy (NST or foliar spray) is most likely at the first sample date, because physiological and environmental factors (e.g., plant metabolism, photodegradation, offsite movement) would have the least amount of time to reduce the effective insecticide concentration present in or on leaf tissue. For NSTs, previous studies have shown that peak concentrations (Zhang et al., 2011) and greatest efficacy against F. fusca (D’Ambrosio et al., 2018) occur when seedlings are young, and decrease in concentration/efficacy as they age. For foliar insecticides, the largest reduction in pest numbers relative to a non-chemical control is also most probable shortly after application. Consequently, comparing larval numbers on insecticide-treated seedlings relative to untreated seedlings at the first sample date provides the most insight into how insecticide resistance status affects insecticide efficacy.

Here, comparisons of mean larval numbers at the first sample date showed that all NSTs tested were not significantly different from the untreated control (Table 5). All non-neonicotinoid insecticides tested with the exception of abamectin (i.e., foliar sprays) had significantly fewer larvae than the untreated control. Together, these results suggest that in the presence of resistant populations, NSTs performed at an equivalent level as an untreated plant. Clearly, F. fusca neonicotinoid resistance status is important when evaluating the efficacy of an insecticide application, and provides context for the data on larval numbers and plant health measures (i.e., leaf count, seedling injury) obtained across the susceptibility period. With the
emerging *F. fusca* resistance issue and ongoing use of NSTs in cotton, accurately determining the economic value of these technologies will be important to shape IRM recommendations designed to mitigate *F. fusca* resistance moving forward.

4 CONCLUSION

Neonicotinoid-resistant *F. fusca* will present a serious issue for cotton growers moving forward. Despite the presence of viable alternatives, neonicotinoid insecticides have continued to be recommended in the region as a prophylactic protection from *F. fusca* injury (Brown 2017, North et al., 2018, Stewart 2016), likely leading to continued erosion of NST efficacy where resistance occurs and potential economic losses for the grower. Clearly, the integration of a broader suite of materials into pest management programs targeting thrips will be important to maintain safe and sustainable early season *F. fusca* control. In this study, we demonstrated that properly timed foliar insecticide applications had superior efficacy on neonicotinoid-resistant *F. fusca* when compared to a NST. While other studies have shown *F. fusca* efficacy with these insecticides, our study also considers resistance status when estimating performance of NSTs and alternative insecticide options against neonicotinoid resistant thrips populations. Accounting for neonicotinoid resistance will be crucial to make accurate *F. fusca* management recommendations as neonicotinoid resistance becomes more widespread in the US Cotton Belt.

DISCLOSURE OF INTEREST

Bayer CropScience, which holds the rights for Stoneville cotton seeds, Gaucho 600FS, and Aeris, provided support for these field studies. BASF, which held no rights to any seed or materials
used at the time of this work, funded the graduate education of D.A. D’Ambrosio from 2016-2018.

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**TABLES**

**Table 1:** Planting and sampling dates for both trial years

<table>
<thead>
<tr>
<th>Trial Year</th>
<th>Planting</th>
<th>Spray application</th>
<th>First sample</th>
<th>Second sample</th>
<th>Third sample</th>
<th>Stand count</th>
<th>Lint harvest</th>
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<td>10 May</td>
<td>28 May</td>
<td>6 June</td>
<td>13 June</td>
<td>20 June</td>
<td>27 June</td>
<td>2 Nov</td>
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<tr>
<td>2017</td>
<td>9 May</td>
<td>26 May</td>
<td>1 June</td>
<td>8 June</td>
<td>15 June</td>
<td>20 June</td>
<td>6 Oct</td>
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Table 2: List of insecticidal treatments used in the field trials

<table>
<thead>
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<th>Active ingredient(s)</th>
<th>Application method</th>
<th>Trade name</th>
<th>IRAC MoA† group</th>
<th>Manufacturer</th>
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<th>Rate Al seed ¹</th>
<th>g Al ha⁻¹</th>
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</thead>
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<td>thiamethoxam†</td>
<td>Seed treatment</td>
<td>Cruiser 5FS</td>
<td>4A- nicotinic acetylcholine receptor (nAChR) competitive modulator</td>
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<td>0.375 mg</td>
<td>53.8</td>
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<td>Seed treatment</td>
<td>Aeris</td>
<td>4A + 1A-acetylcholinesterase (AChE) inhibitor</td>
<td>Bayer CropScience LP, RTP, NC</td>
<td>0.18 L§</td>
<td>0.375 mg each of imidacloprid and thiodicarb</td>
<td>53.8 each of imidacloprid and thiodicarb</td>
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<td>Orthene 97</td>
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<td>Dow AgroSciences, LLC, Indianapolis, IN</td>
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<td>Foliar spray</td>
<td>Agri-Mek SC</td>
<td>6- glutamate-gated chloride channel allosteric modulator</td>
<td>Syngenta Crop Protection, LLC, Greensboro, NC</td>
<td>0.25 L</td>
<td>-</td>
<td>20.7</td>
</tr>
<tr>
<td>cyantraniliprole</td>
<td>Foliar spray</td>
<td>Exirel</td>
<td>28- ryanodine receptor modulator</td>
<td>E. I. du Pont de Nemours and Co., Wilmington, DE</td>
<td>1.0 L</td>
<td>-</td>
<td>100.0</td>
</tr>
<tr>
<td>cyantraniliprole + abamectin</td>
<td>Foliar spray</td>
<td>Minecto Pro</td>
<td>28 + 6</td>
<td>Syngenta Crop Protection, LLC, Greensboro, NC</td>
<td>0.73 L</td>
<td>-</td>
<td>98.6 cyantraniliprole and 20.7 abamectin</td>
</tr>
</tbody>
</table>

† Insecticide resistance action committee modes of action, available at http://www.irac-online.org
‡ Materials also used in LC₅₀ bioassays to determine *F. fusca* resistance status
§ Liters formulated product ha⁻¹ was calculated using seed stocking density of 143,518 seeds ha⁻¹
### Table 3: Correlation analysis of the four plant vigor metrics measured in the trial

<table>
<thead>
<tr>
<th>Vigor metric</th>
<th>Plant height</th>
<th>Wet weight</th>
<th>Dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet weight</td>
<td>0.89 (2.7e-23)**</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dry weight</td>
<td>0.53 (6.3e-6)***</td>
<td>0.60 (1.4e-7)***</td>
<td>-</td>
</tr>
<tr>
<td>True leaf count</td>
<td>0.55 (2e-6)***</td>
<td>0.71 (5.4e-11)***</td>
<td>0.37 (0.0024)**</td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.01, ***p<0.001
Table 4: Type III effects for insecticide performance metrics evaluated in the field trials

<table>
<thead>
<tr>
<th>Metric</th>
<th>Parameter</th>
<th>Numerator degrees of freedom</th>
<th>Denominator degrees of freedom</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Larval numbers</strong></td>
<td>Insecticide treatment (IT)</td>
<td>7</td>
<td>220.6</td>
<td>14.07</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td></td>
<td>Cotton variety (CV)</td>
<td>1</td>
<td>220.6</td>
<td>7.54</td>
<td>0.0065**</td>
</tr>
<tr>
<td></td>
<td>Days after planting (DAP)</td>
<td>1</td>
<td>299</td>
<td>15.60</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td></td>
<td>IT x CV</td>
<td>7</td>
<td>220.6</td>
<td>0.34</td>
<td>0.9355</td>
</tr>
<tr>
<td></td>
<td>IT x DAP</td>
<td>7</td>
<td>299</td>
<td>4.45</td>
<td>0.0001***</td>
</tr>
<tr>
<td></td>
<td>CV x DAP</td>
<td>1</td>
<td>299</td>
<td>3.40</td>
<td>0.0663</td>
</tr>
<tr>
<td></td>
<td>IT x CV x DAP</td>
<td>7</td>
<td>299</td>
<td>0.29</td>
<td>0.9560</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>1</td>
<td>5.939</td>
<td>1.38</td>
<td>0.2854</td>
</tr>
<tr>
<td><strong>Expanded true leaf count</strong></td>
<td>Insecticide treatment (IT)</td>
<td>7</td>
<td>1770</td>
<td>14.98</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td></td>
<td>Cotton variety (CV)</td>
<td>1</td>
<td>1770</td>
<td>76.88</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td></td>
<td>Days after planting (DAP)</td>
<td>1</td>
<td>1410</td>
<td>8133.34</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td></td>
<td>IT x CV</td>
<td>7</td>
<td>1770</td>
<td>1.23</td>
<td>0.2801</td>
</tr>
<tr>
<td></td>
<td>IT x DAP</td>
<td>7</td>
<td>1410</td>
<td>4.41</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td></td>
<td>CV x DAP</td>
<td>1</td>
<td>1410</td>
<td>0.29</td>
<td>0.5905</td>
</tr>
<tr>
<td></td>
<td>IT x CV x DAP</td>
<td>7</td>
<td>1410</td>
<td>0.68</td>
<td>0.6876</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>1</td>
<td>5.988</td>
<td>3.14</td>
<td>0.1268</td>
</tr>
<tr>
<td><strong>Seed cotton yield†</strong></td>
<td>Insecticide treatment (IT)</td>
<td>7</td>
<td>105</td>
<td>0.63</td>
<td>0.7326</td>
</tr>
<tr>
<td></td>
<td>Cotton variety (CV)</td>
<td>1</td>
<td>105</td>
<td>0.56</td>
<td>0.4576</td>
</tr>
<tr>
<td></td>
<td>IT x CV</td>
<td>7</td>
<td>105</td>
<td>1.41</td>
<td>0.2105</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>1</td>
<td>6</td>
<td>62.43</td>
<td>0.0002***</td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.01, ***p<0.001
†- Since yield is collected at a single time point at the end of the season, days after planting (DAP) and subsequent interactions are not applicable parameters when analyzing this metric.
Table 5: Mean larvae per 8 seedlings for each insecticide treatment across both field trial years

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment type</th>
<th>Mean larvae/8 seedlings</th>
<th>Difference between treatment and untreated mean</th>
<th>Simultaneous 95% confidence limits (lower, upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated (fungicide-only)</td>
<td>-</td>
<td>62.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>Seed treatment</td>
<td>78.3</td>
<td>15.9</td>
<td>-19.1, 50.8</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>Seed treatment</td>
<td>51.1</td>
<td>11.3</td>
<td>-46.2, 23.7</td>
</tr>
<tr>
<td>imidacloprid + thiodicarb</td>
<td>Seed treatment</td>
<td>64.1</td>
<td>1.7</td>
<td>-33.2, 36.7</td>
</tr>
<tr>
<td>Acephate</td>
<td>Foliar spray</td>
<td>26.1*</td>
<td>-36.3</td>
<td>-71.3, -1.4</td>
</tr>
<tr>
<td>Spinetoram</td>
<td>Foliar spray</td>
<td>20.6*</td>
<td>-41.8</td>
<td>-76.7, -6.8</td>
</tr>
<tr>
<td>Abamectin</td>
<td>Foliar spray</td>
<td>57.5</td>
<td>-4.9</td>
<td>-39.9, 30.0</td>
</tr>
<tr>
<td>Cyantraniliprole</td>
<td>Foliar spray</td>
<td>9.5*</td>
<td>-52.9</td>
<td>-87.9, -18.0</td>
</tr>
<tr>
<td>cyantraniliprole + abamectin</td>
<td>Foliar spray</td>
<td>12.7*</td>
<td>-49.7</td>
<td>-84.7, -14.9</td>
</tr>
</tbody>
</table>

* - Mean larval numbers are significantly different from untreated at α=0.05 by Dunnett’s test
Figure 1: Average *F. fusca* larvae per 8 seedlings for all cotton variety x insecticide treatment combinations across both trials. Means separations by Tukey’s HSD. Bars followed by similar letters do not significantly differ at α=0.05.
Figure 2: Average expanded (width ≥ 10mm) true leaf count per seedling for all cotton variety x insecticide treatment combinations across both trials. Means separations by Tukey’s HSD. Bars followed by similar letters do not significantly differ at α=0.05.
SUPPLEMENTAL INFORMATION

Untreated seedling exclusion rationale

Untreated seedlings were heavily injured by thrips early in the season, stunting their growth, and causing them to be noticeably smaller than their treated counterparts as the season progressed (Figure S1). Later-arriving *F. fusca* may be more likely to encounter the comparatively larger, treated plants instead of the smaller, untreated plants. Such a trend is an artifact of the field trial setting, as commercial fields with a uniform thrips management strategy would tend to have more uniformly-sized plants, making insect encounters with a particular plant more random. This caused oddities such as reduced numbers of larvae and eggs on older untreated seedlings relative to treated plants (Figure S2), and had the potential to cause statistically significant interactions with treatment (i.e. larval numbers on insecticide-treated plants tended to remain constant or decline at the end of the trial when thrips flights would be expected to decrease, but decreased consistently in untreated plants (Figure S2)) that may obfuscate the ability to recover meaningful differences in insecticide performance. Data from all seedlings, including the untreated, can be found in this supplemental information (Table S1).
**SUPPLEMENTAL TABLES**

**Table S1:** Larval numbers, expanded true leaf count, and injury rating means (with standard error) for all treatments and both cotton varieties for both field trial years

<table>
<thead>
<tr>
<th>Year</th>
<th>Sample</th>
<th>Variety</th>
<th>Treatment</th>
<th>Larvae per 8 seedlings</th>
<th>Expanded true leaf count (&gt;10mm)</th>
<th>Injury rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>1</td>
<td>ST4946</td>
<td>Untreated</td>
<td>53.75 ± 7.01</td>
<td>1.775 ± 0.08</td>
<td>3.25 ± 0.16</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST4946</td>
<td>Thiamethoxam</td>
<td>54.5 ± 8.17</td>
<td>2 ± 0</td>
<td>2.75 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST4946</td>
<td>Imidacloprid</td>
<td>22.5 ± 4.91</td>
<td>2.2 ± 0.09</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST4946</td>
<td>imidacloprid + thiodicarb</td>
<td>33.25 ± 4.23</td>
<td>2.4 ± 0.13</td>
<td>1.5 ± 0.29</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST4946</td>
<td>Acephate</td>
<td>13.25 ± 2.78</td>
<td>2.1 ± 0.1</td>
<td>2.25 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST4946</td>
<td>Spinetoram</td>
<td>9.25 ± 3.35</td>
<td>2.55 ± 0.11</td>
<td>1.5 ± 0.29</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST4946</td>
<td>Abamectin</td>
<td>19.5 ± 4.09</td>
<td>2.25 ± 0.1</td>
<td>2.25 ± 0.48</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST4946</td>
<td>Cyantraniliprole</td>
<td>9.75 ± 3.2</td>
<td>2.3 ± 0.11</td>
<td>2 ± 0.41</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST4946</td>
<td>cyantraniliprole + abamectin</td>
<td>13.5 ± 2.22</td>
<td>2.3 ± 0.13</td>
<td>2 ± 0</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST6448</td>
<td>Untreated</td>
<td>42.375 ± 5.75</td>
<td>1.45 ± 0.09</td>
<td>3.25 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST6448</td>
<td>Thiamethoxam</td>
<td>21.75 ± 4.33</td>
<td>1.5 ± 0.14</td>
<td>2 ± 0.41</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST6448</td>
<td>Imidacloprid</td>
<td>16.75 ± 2.46</td>
<td>1.9 ± 0.07</td>
<td>1.33 ± 0.33</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST6448</td>
<td>imidacloprid + thiodicarb</td>
<td>18.75 ± 3.61</td>
<td>1.9 ± 0.07</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST6448</td>
<td>Acephate</td>
<td>10.75 ± 3.01</td>
<td>1.9 ± 0.07</td>
<td>2 ± 0</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST6448</td>
<td>Spinetoram</td>
<td>5.75 ± 0.63</td>
<td>1.9 ± 0.1</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST6448</td>
<td>Abamectin</td>
<td>12.75 ± 1.38</td>
<td>2 ± 0.07</td>
<td>2.25 ± 0.63</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST6448</td>
<td>Cyantraniliprole</td>
<td>4.25 ± 0.63</td>
<td>2.05 ± 0.05</td>
<td>1.25 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>1</td>
<td>ST6448</td>
<td>cyantraniliprole + abamectin</td>
<td>7.25 ± 2.78</td>
<td>2 ± 0</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST4946</td>
<td>Untreated</td>
<td>28.5 ± 9.88</td>
<td>2.55 ± 0.36</td>
<td>3.75 ± 0.16</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST4946</td>
<td>Thiamethoxam</td>
<td>59.25 ± 5.65</td>
<td>4.35 ± 0.25</td>
<td>3.5 ± 0.29</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST4946</td>
<td>Imidacloprid</td>
<td>35.75 ± 5.01</td>
<td>4.95 ± 0.28</td>
<td>2 ± 0.37</td>
</tr>
<tr>
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<td>2</td>
<td>ST4946</td>
<td>imidacloprid + thiodicarb</td>
<td>41.75 ± 4.23</td>
<td>4.6 ± 0.2</td>
<td>2.25 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST4946</td>
<td>Acephate</td>
<td>21.25 ± 3.97</td>
<td>5.35 ± 0.18</td>
<td>2.25 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST4946</td>
<td>Spinetoram</td>
<td>20.75 ± 4.01</td>
<td>5.45 ± 0.14</td>
<td>1.75 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST4946</td>
<td>Abamectin</td>
<td>26 ± 1.96</td>
<td>4.9 ± 0.2</td>
<td>2.25 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST4946</td>
<td>Cyantraniliprole</td>
<td>21.75 ± 3.57</td>
<td>4.95 ± 0.18</td>
<td>3 ± 0</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST4946</td>
<td>cyantraniliprole + abamectin</td>
<td>26.5 ± 3.4</td>
<td>4.9 ± 0.18</td>
<td>2.5 ± 0.29</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST6448</td>
<td>Untreated</td>
<td>19 ± 7.75</td>
<td>1.75 ± 0.29</td>
<td>3.875 ± 0.13</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST6448</td>
<td>Thiamethoxam</td>
<td>40.5 ± 5.61</td>
<td>3.6 ± 0.18</td>
<td>2.5 ± 0.29</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST6448</td>
<td>Imidacloprid</td>
<td>36 ± 7.85</td>
<td>3.5 ± 0.14</td>
<td>1.67 ± 0.33</td>
</tr>
<tr>
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<td>2</td>
<td>ST6448</td>
<td>imidacloprid + thiodicarb</td>
<td>34.5 ± 3.1</td>
<td>3.6 ± 0.11</td>
<td>1.25 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST6448</td>
<td>Acephate</td>
<td>12.75 ± 2.39</td>
<td>3.5 ± 0.15</td>
<td>2.25 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST6448</td>
<td>Spinetoram</td>
<td>20 ± 8.63</td>
<td>3.9 ± 0.14</td>
<td>1.25 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST6448</td>
<td>Abamectin</td>
<td>19.25 ± 3.07</td>
<td>4.45 ± 0.15</td>
<td>2.25 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST6448</td>
<td>Cyantraniliprole</td>
<td>22 ± 6.34</td>
<td>3.95 ± 0.09</td>
<td>1.75 ± 0.48</td>
</tr>
<tr>
<td>2016</td>
<td>2</td>
<td>ST6448</td>
<td>cyantraniliprole + abamectin</td>
<td>20.5 ± 6.12</td>
<td>3.85 ± 0.24</td>
<td>1.5 ± 0.29</td>
</tr>
<tr>
<td>Year</td>
<td>Week</td>
<td>Substrate</td>
<td>Treatment</td>
<td>Mortality Rate</td>
<td>Inhibition Rate</td>
<td>Mortality 1</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>-----------</td>
<td>-----------</td>
<td>---------------</td>
<td>----------------</td>
<td>------------</td>
</tr>
<tr>
<td>2016</td>
<td>3</td>
<td>ST4946</td>
<td>Untreated</td>
<td>14.125 ± 6.59</td>
<td>2.925 ± 0.48</td>
<td>3 ± 0</td>
</tr>
<tr>
<td>2016</td>
<td>3</td>
<td>ST4946</td>
<td>Thiamethoxam</td>
<td>40.25 ± 6.18</td>
<td>6.25 ± 0.2</td>
<td>2.5 ± 0.29</td>
</tr>
<tr>
<td>2016</td>
<td>3</td>
<td>ST4946</td>
<td>Imidacloprid</td>
<td>15.75 ± 0.75</td>
<td>6.7 ± 0.16</td>
<td>1.5 ± 0.29</td>
</tr>
<tr>
<td>2016</td>
<td>3</td>
<td>ST4946</td>
<td>imidacloprid + thiodicarb</td>
<td>29.75 ± 8.26</td>
<td>7 ± 0.15</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>2016</td>
<td>3</td>
<td>ST4946</td>
<td>Acephate</td>
<td>21.75 ± 11.48</td>
<td>7 ± 0.18</td>
<td>2 ± 0.41</td>
</tr>
<tr>
<td>2016</td>
<td>3</td>
<td>ST4946</td>
<td>Spinetoram</td>
<td>11.25 ± 4.23</td>
<td>6.7 ± 0.19</td>
<td>0.75 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>3</td>
<td>ST4946</td>
<td>imidacloprid + thiodicarb</td>
<td>15.75 ± 0.75</td>
<td>6.7 ± 0.24</td>
<td>1.25 ± 0.25</td>
</tr>
<tr>
<td>2016</td>
<td>3</td>
<td>ST4946</td>
<td>Acephate</td>
<td>21.75 ± 11.48</td>
<td>7 ± 0.18</td>
<td>2 ± 0.41</td>
</tr>
<tr>
<td>2016</td>
<td>3</td>
<td>ST4946</td>
<td>Spinetoram</td>
<td>11.25 ± 4.23</td>
<td>6.7 ± 0.19</td>
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Table S1 (continued from 124)
Table S1 (continued from 125)

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Figure S1: Mean plant height for all three treatment types across sample days for both cotton varieties in both trial years.
Figure S2: Average thrips larvae per plant for all three treatment types across sample days and cotton varieties in both trial years.
CONCLUSION

This work expands the understanding of thrips behavior in two agroecosystems. In Chapter 1, the role of imidacloprid and cyantraniliprole are better understood in terms of how they modulate thrips feeding to disrupt virus transmission in tomato. While their differing modes of action of these insecticides were already understood, our study revealed that they also have different effects on the ultimate feeding behavior of *F. fusca*. Insecticides can reduce virus transmission by sharply decreasing the frequency of feeding events, as in the case of imidaclorpid, but can also reduce virus transmission by altering the transmission likelihood of a particular feeding event, even if the frequency of feeding events is less drastically reduced, as in the case of cyantraniliprole. As new systemic insecticide candidates are developed and evaluated for use in TSW management, understanding that a compound can act via multiple avenues of feeding behavioral modulation to ultimately reduce virus spread can ensure they are properly and fully evaluated.

In cotton, multiple aspects of the *F. fusca*-cotton seedling-NST interaction are better understood. Chapter 2 reveals that the efficacy of NSTs decreases over time as the plant grows larger, allowing thrips to establish more larvae on older plants. Susceptible and resistant thrips alike are able to take advantage of this decrease in efficacy, with resistant thrips more readily exploiting this decrease. This could allow thrips to have access to an insecticide-treated plant as a reproductive resource, thereby encouraging low dose exposure to neonicotinoids. Over time, these cycles of low dose exposure could select for resistance, and could in part explain the widespread resistance to these compounds seen in the field today. While our study evaluates a thrips population with a level of resistance higher than the laboratory-raised, neonicotinoid-
naïve population, evaluations of other *F. fusca* populations in the field have discovered populations with much higher LC₅₀s. Assumedly, highly resistant *F. fusca* would not demonstrate a time-related response to changing NST efficacy, as they could exploit young seedlings with the highest concentration of systemic insecticide present. As such, our resistant population probably represents a *F. fusca* population in “transition,” wherein larger percentages of resistant individuals are present in the population, but there is still a degree of heterogeneity present in terms of resistant status. Future studies evaluating a more homogeneous, highly resistant population would determine if this pattern of low dose exposure could ultimately select for a population that is completely unaffected by NSTs.

Chapter 3 reveals that *F. fusca* has a preference for cotyledon tissue when ovipositing. Systemic neonicotinoids delivered via seed treatment tend to concentrate in the cotyledons. This offers an explanation of why NSTs were so effective in managing *F. fusca* for many years, as the behavior of *F. fusca*, coupled with the distribution patterns of the insecticide, ensured high levels of insecticide exposure. However, we also discovered that *F. fusca* can modulate their oviposition behavior and oviposit on true leaf tissue, where the concentration of insecticide is putatively lower. Similar to the temporal reductions in NST efficacy documented in Chapter 2, this behavioral shift can allow insects to access insecticide-treated plants as a reproductive resource, and could facilitate low dose exposure, thereby driving resistance. Our study demonstrates that treated cotyledon avoidance is lost in resistant populations, allowing them to oviposit on the cotyledons of young, NST-grown seedlings, and ultimately establish economically injurious populations of larvae. In addition, this chapter shows that larval distribution within the seedling is more uniform than oviposition, suggesting that thrips
disproportionally hatch on the cotyledons, but migrate elsewhere on the plant, where they can cause characteristic patterns of thrips injury such as growth point malformation. Overall, these results show that for future systemic *F. fusca* management tools, whether insecticide, plant-incorporated protectant, or host plant resistance mechanism, cotyledon expression is important. Dissimilar expression throughout the plant could provide an avenue for *F. fusca* to overcome the strategy, and over time could lead to resistance. The more uniform distribution of larval thrips provides insight onto the feasibility of foliar insecticide sprays in managing this pest, and suggests that well-timed sprays that coincide with the expansion of the first true leaf would knock back thrips populations hatching on the cotyledons, thereby preventing them from injuring the developing true leaves of the plant. This knowledge will be important in developing the next generation of thrips control tools in cotton as NSTs continue to fail.

In Chapter 4, the feeding behavior of resistant versus susceptible *F. fusca* on NST-grown cotton cotyledons was determined. It showed that NSTs reduce the number of feeding events in the susceptible population, but do not in the resistant populations. These results mirror the findings of Chapter 3, and suggest that adult *F. fusca* utilize this feeding interaction to determine the suitability of a seedling as an oviposition substrate; susceptible thrips perceive the insecticide as a deterrent, cease feeding, and ultimately do not oviposit, whereas resistant thrips feed normally on these plants, and ultimately oviposit normally as well. Additional studies have shown that NSTs have a nonlethal effect on adult thrips, which could limit their selection pressure in the field and increase their useful life in such a setting. Altogether, these results show the utility of deterrents in thrips management in cotton. Like the results of previous chapters, this information can inform future thrips management strategies. Nonlethal
deterrents, provided they are presented where the thrips is likely to encounter them (Chapter 3), and with limited temporal/spatial variations in presentation (Chapters 2 and 3), could provide the previously seen efficacy of NSTs, without the weaknesses that thrips can exploit.

While the applied behavioral studies in Chapters 2, 3, and 4 provide insight into future thrips management tactics in cotton, several foliar insecticide sprays with demonstrated thrips activity and non-neonicotinoid modes of action are currently available. These sprays could provide more immediate relief to cotton producers contending with resistant thrips populations, and/or serve as the next primary thrips management tactic in cotton. Based on behavioral findings on *F. fusca* oviposition discussed in Chapter 3, these sprays, if timed with the emergence of the first true leaf, could effectively knock back populations of injurious thrips larvae as they eclosed from the cotyledons, thereby protecting the developing true leaves. In Chapter 5, we evaluate a few of these chemistries in small field trial studies alongside existing NST technologies in trials naturally infested by neonicotinoid-resistant *F. fusca*. The results showed these well-timed foliar sprays, namely spinetoram and cyantraniliprole, outperformed NSTs in terms of reducing the number of thrips larvae on seedlings, and were comparable to NSTs in terms of promoting plant vigor. Inclusion of these foliar sprays in a thrips management program would diversify the modes of action available to cotton producers, thereby staving off the development of resistance. Further, they could feasibly provide solutions to growers contending with resistant *F. fusca* populations in the near term. Future thrips behavioral studies on these foliar sprays, similar to the ones discussed in Chapters 2, 3, and 4, could go a long way in determining any potential weaknesses in these strategies that *F. fusca* could exploit.
Overall, these works demonstrate the value of applied behavioral studies when developing pest management strategies. While these works demonstrate the value of thrips studies in tomato and cotton, such findings could be of interest to multiple insect pest-crop systems. By bridging the gap between an insecticide’s molecular-level mode of action and its ultimate effect on crop production in the field, these studies elucidate “how” the insecticide works at the level of the individual insect. These studies can provide hints as to how the insect can overcome an insecticide in a field setting, allowing for such weaknesses to be addressed when developing future tools for management. Other management tools beyond insecticides, such as resistant plant cultivars and genetically modified crops, can benefit similarly. Obtaining more complete understandings of the insect-plant-crop protectant interaction will be paramount in addressing the future challenges of sustainable pest management and agricultural production moving forward.