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Evaluation of alternative mode of action insecticides in managing neonicotinoid-resistant *Frankliniella fusca* in cotton

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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.

1. Introduction

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, *Frankliniella fusca* (Hinds) is the most important early season insect pest of cotton (Cook et al., 2011). Adult *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 *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 *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 *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, 2014, 2016). However, in recent years, reports of reduced NST control led to the discovery of widespread resistance to imidacloprid and thiamethoxam NSTs in *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 2014, 2016; Stewart, 2016), 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.

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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 *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 cotton production in North Carolina were used (Edmisten et al., 2018).

Table 1
Planting and sampling dates for both trial years.

Trial Year	Planting	Spray application	First sample	Second sample	Third sample	Stand count	Harvest
2016	10 May	28 May	6 June	13 June	20 June	27 June	2 Nov
2017	9 May	26 May	1 June	8 June	15 June	20 June	6 Oct

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 placed 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 (LC_{50}) 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 LC_{50} levels calculated for the field-collected *F. fusca* population. LC_{50} 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) (Huseth et al., 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, EverGol® 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 CO_2 powered backpack sprayer connected to a spray boom consisting of two flat fan spray nozzles (TR8002VS, TeeJet Technologies, Wheaton, IL, USA) spaced 0.9 m apart calibrated to deliver foliar sprays at a rate of 93.54 L ha^{-1}. Nozzles were centered

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