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Nonanal, a new fall armyworm sex pheromone component, significantly increases the efficacy of pheromone lures

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Abstract

Background: The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), is a global pest that feeds on >350 plant species and severely limits production of cultivated grasses, vegetable crops and cotton. An efficient way to detect new invasions at early stages, and monitor and quantify the status of established infestations of this pest is to deploy traps baited with species-specific synthetic sex pheromone lures.

Results: We re-examined the compounds in the sex pheromone glands of FAW females by gas chromatography-electroantennogram detector (GC-EAD), GC–mass spectrometry (MS), behavioral and field assays. A new bioactive compound from pheromone gland extracts was detected in low amounts (3.0% relative to (*Z*)-9-tetradecenyl acetate (Z9-14:OAc), the main pheromone component), and identified as nonanal. This aldehyde significantly increased attraction of male moths to a mix of Z9-14:OAc and (*Z*)-7-dodecenyl acetate in olfactometer assays. Adding nonanal to this two-component mix also doubled male trap catches relative to the two-component mix alone in cotton fields, whereas nonanal alone did not attract any moths. The addition of nonanal to each of three commercial pheromone lures also increased male catches by 53–135% in sorghum and cotton fields.

Conclusion: The addition of nonanal to pheromone lures should improve surveillance, monitoring and control of FAW populations.

Supporting information may be found in the online version of this article.

Keywords: *Spodoptera frugiperda*; fall armyworm; sex pheromone; nonanal; lures; trapping

1 INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a highly polyphagous cosmopolitan pest that feeds on >350 plant species from 76 plant families.[1] The FAW prefers plants in the Poaceae (106 species), but larvae cause significant damage to plants in other families including Malvaceae, Fabaceae, Brassicaceae, Solanaceae, Amaryllidaceae and Amaranthaceae. Major host plants include maize, rice, sorghum, sugarcane, wheat, buckwheat, barley, oat, millet, cotton, peanut, alfalfa and various vegetable crops,[2] making it one of the most important global agricultural pests.

Endemic to the Western Hemisphere, the FAW completes multiple generations year-round from northern Argentina to southern Florida and Texas.[3] The FAW was first detected in West Africa in early 2016, and by late 2018, it spread through the entire sub-Saharan region. In Africa, populations of this pest have increased exponentially in the last 4 years, with significant economic impacts, especially to small-scale farmers and consumers. The FAW can cause yield losses in maize of 22–53% or an equivalent

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This paper is dedicated to the memory of Dr James Tumlinson, a pioneer in the identification of semiochemicals that mediate insect–insect and insect–plant interactions, and their biosynthetic pathways.

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of 8.3–20.6 million tons per year at a value of US$2.5–6.2 billion, with an additional US$13 billion worth of crops at risk.[4] In 2018 it became established in Yemen, the Indian subcontinent, Bangladesh, Thailand, Myanmar and Sri Lanka, and in 2020 it spread into most of China and Australia, causing devastating crop losses in major crop production systems. International agencies (e.g. Food and Agriculture Organization of the United Nations) consider control of the FAW an international priority because of the wide range of its host plants, high fecundity, several generations in a single season in its invasive tropical habitats, strong-flying and dispersal capability, rapid evolution of resistance to insecticides, and extensive use of hazardous pesticides for its management; overall, the FAW has become endemic in its invasive range, with devastating effects.[2]

An efficient way to detect new infestations at very early stages, monitor the size of established populations, and suppress resurgent infestations of this and other agricultural pests is to deploy traps with synthetic lures that mimic the highly species-specific female sex pheromone.[5,6] Although effective in comparison to other lures (e.g. light traps, floral scents), sex pheromone lures can be improved to attract more FAW males and fewer nontarget species that look like the FAW, and therefore can confuse farmers.[7,8] For example, in Africa the FAW is difficult to distinguish from other lepidopteran pests, such as African armyworm (*Spodoptera exempta*), beet armyworm (*Spodoptera exigua*), African cotton leafworm (*Spodoptera littoralis*) and various *Helicoverpa* species in the same family (Noctuidae). In the United States, FAW traps also capture *Leucania phragmatidicola*, in some regions at more than twice the rate of *S. frugiperda*. Development and implementation of coordinated integrated pest management (IPM) programs is highly dependent on effective species-specific detection, monitoring and pest management at the local, state, regional, country and continental levels, and effective pheromone traps are critical cornerstones of IPM programs.

The FAW sex pheromone was identified 37 years ago using pheromone gland extracts and volatiles from pheromone-emitting (calling) females.[9] Although five volatile components were identified from calling females — (*Z*)-9-tetradecenyl acetate (Z9-14:OAc), (*Z*)-7-dodecenyl acetate (Z7-12:OAc), (*Z*)-11-hexadecenyl acetate (Z11-16:OAc), dodecyl acetate (12:OAc), and 11-dodecenyl acetate (11-12:OAc) — only Z9-14:OAc and Z7-12:OAc were needed to effectively attract males.[9] The effectiveness of a binary combination of Z9-14:OAc and Z7-12:OAc also was supported in studies in Costa Rica.[10] Nevertheless, Z9-12:OAc and Z11-16:OAc are commonly added in commercial pheromone lures.

The ratio of sex pheromone components has been debated over the years as well. Tumlinson *et al.*[9] concluded that a release ratio of 96.6:3.4 (equivalent to 100:3.52 relative to Z9-14:OAc) was most effective; the lure (septum) loading was 81.6:0.5 (100:0.61). Indeed, Meagher and Mitchell[11] showed that the two-component blend was highly effective at a loading ratio of 80.3:0.5 (100:0.62). The two-component blend, loaded at 99.42:0.58 (100:0.58; the 'PSU lure'), while not as effective as the four-component lure, caught fewer nontarget *Leucania* (*Mythimna*) moths, which were being confused by farmers and crop scouts with FAW.[7] Likewise, the combination of Z9-14:OAc and Z7-12:OAc (loaded at 99.4:0.6; 100:0.6 relative to Z9-14:OAc) performed best in field trials in Costa Rica, the addition of Z11-16:OAc did not significantly improve the lure[10] and 100:1 was effective in field-trapping experiments in Brazil.[12]

We hypothesized that some sex pheromone components might have been overlooked as a consequence of technical limitations 37 years ago.[9] Additional components could improve the performance of commercial lures, leading to the development of more effective lures meeting international demand. By integrating analytical and field experiments, we identified a new sex pheromone component that significantly improved attraction of FAW males to pheromone lures.

2 MATERIALS AND METHODS

2.1 Insects

Spodoptera frugiperda pupae were originally obtained from Benzon Research (Carlisle, PA, USA) in 2018 and reared on artificial diet (Southland Products, Lake Village, AR, USA) at 27 ± 1 °C and 30–40% relative humidity (RH) in individual plastic cups (30 mL) each capped with a paper lid. Pupae were separated by sex and placed in separate 30 × 30 × 30 cm rearing cages (Bugdorm, MegaView Science, Taiwan) in separate incubators under a reversed 12 h:12 h, light:dark photoperiod. Newly eclosed adults were collected daily and transferred to new cages with access to 10% sucrose in water. All of the laboratory bioassays were done using 3- to 4-day-old moths.

2.2 Pheromone gland extraction

Sex pheromone glands of 3–4-day-old FAW virgin females were dissected from the extruded ovipositors with fine forceps and each set of 10 glands was placed in a conical glass GC insert (300 µL capacity; Limited Volume Insert, Fisher Scientific, Hampton, NH, USA) containing 30 µL hexane (SupraSolv; Millipore Sigma, Burlington, MA, USA). Dissections were conducted 3–4 h after the onset of the scotophase, and after 10 min in hexane, the extract without the glands was transferred to a second GC insert and concentrated to 10 µL under a gentle flow of nitrogen. Six replicates were obtained by repeating this extraction procedure.

2.3 Electrophysiology

We used a gas chromatograph coupled to an electroantennogram detector (GC-EAD) to identify biologically active compounds in the female sex pheromone gland extracts. A male antenna was ablated at the base and inserted into a reference glass electrode filled with Ephrussi and Beadle ringer, whereas the recording electrode was connected to the cut tip of the antenna and connected to a custom-made amplifier.[13] The amplifier was connected to a G3456-60010 AIB board in a 7890 GC (Agilent Technologies, Palo Alto, CA, USA) which synchronized the outputs of the FID and EAD. The GC was equipped with a DB-WAXetr column (30 m × 0.25 mm × 0.25 µm; Agilent Technologies) and operated in splitless mode. Hydrogen was used as the carrier gas at an average linear velocity of 45 cm s^{-1}. The oven program was set to 40 °C for 2 min, increased at 10 °C min^{-1} to 250 °C and held for 15 min. The FID was set at 280 °C. The capillary column was split 1:1 between the FID and the EAD using a microfluidics effluent splitter with makeup gas (G3180-90120; Agilent Technologies). The effluent capillary for the EAD passed through a modified MS transfer line set at 270 °C, and into a custom-made water-cooled glass odor delivery tube (30 cm × 8 mm) set at 19 °C, where it was mixed with humidified medical grade air (500 mL/min). The male antenna was positioned 0.5 cm from the outlet of the odor delivery tube. The odor delivery tube, EAD amplifier and microscope were housed within a Faraday cage.

An aliquot of 1 μL pheromone gland extract (1 female-equivalent) was injected in the GC-EAD. Clean hexane that was handled in the same manner, but without glands, was used as control. Each male was used only once and the GC-EAD was replicated with six antennae from six males ($n = 6$).

2.4 Identification of bioactive compounds

Gland extracts were analyzed on a GC–mass spectrometry (MS) apparatus (6890 GC and 5975 MS; Agilent Technologies) operated in pulsed splitless mode (15 psi for 0.5 min, then 6 psi) and equipped with a DB-WAXetr column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies), with helium as the carrier gas at an average velocity of 34 cm/s. The oven program was set to 40 °C for 2 min, increased at 10 °C min^{-1} to 250 °C and held for 15 min. Injector temperature was 250 °C, transfer line was 260 °C and the MS quadrupole was 150 °C. The mass-to-charge ratio range was from 33 to 650. Compounds were identified based on their Kovats indices, electron ionization mass spectra, and comparison and coinjection with synthetic standards.

2.5 Behavioral assays

A linear olfactometer was used to test the behavior of FAW males to different blends. The olfactometer consisted of a Plexiglas tube [6.5 cm inner diameter (i.d.), 145 cm long] that was connected at the downwind end to an in-line air pump that generated an internal airflow of 0.1 m s^{-1}. The pump was connected to a tube that exhausted into a fume hood and out of the building. A 20-cm-long Plexiglas tube filled with activated charcoal was connected to the upwind end of the olfactometer to filter the ambient air entering the olfactometer. A virgin male moth (3–4 days old) that did not have any contact with female moths was placed in a release cage at the downwind end of the olfactometer and allowed to acclimate for 3–4 min to the air flow. Four mixes of Z9-14:OAc (Bedoukian Research, Danbury, CT, USA), Z7-12:OAc (a gift from Dr Kenneth Haynes) and nonanal (98%; Millipore Sigma) were prepared in hexane (Table 1). The concentrations of Z9-14:OAc and Z7-12:OAc in each mix were 10 and 0.058 ng μL^{-1}, respectively. Different amounts of nonanal (0, 0.05, 0.1 and 1%) were added to the two-component mix and all the doses were tested on each day in a randomized complete block design to control for day and dose effects ($n = 20$ per dose). An aliquot of 10 μL was added to a piece of filter paper (Whatman #1; GE Healthcare Life Sciences, Chalfont St Giles, UK) and the solvent was left to evaporate for 10 min at room temperature. The filter paper was introduced through a 2-cm hole at the upwind end of the olfactometer, 4 cm from the charcoal filter. Each filter paper was tested only once and discarded. We tested each male moth for 5 min and the sequence of its behavioral responses was recorded as follows: activation (wing fanning and start moving upwind), flying upwind (reaching the half-way point of the olfactometer) and contact (landing on the filter paper). Each male moth was used only once in the bioassay and all of the experiments were conducted 4–7 h into the scotophase under a dark-room red safelight, illuminated from 1 m above the olfactometer.

2.6 Field experiments

We tested the effects of adding nonanal to sex pheromone lures with field populations of FAW. Two hundred red rubber septa (11 mm; Wheaton, Millville, NJ, USA) were ultrasonicated for 6 h in hexanes (EMSURE, ACS; Millipore Sigma) in a capped 1-L glass bottle at room temperature. The solvent then was discarded and the procedure was repeated. Septa were allowed to dry on aluminum foil in a fume hood for 48 h. An aliquot of 100 μL containing 1000 μg Z9-14:OAc and 5.8 μg Z7-12:OAc in hexane was loaded into each rubber septum and left in a fume hood for 15 h to evaporate the hexane. Aliquots of 10, 20 and 40 μg nonanal, corresponding to 1%, 2% and 4% of the amount of Z9-14:OAc, were each diluted in 100 μL paraffin oil (MP Biomedicals, Solon, OH, USA), and loaded in a separate dispenser that was placed next to each pheromone septum (Table 2). The nonanal dispenser was adapted from a previous design[14] and consisted of a 2-mL borosilicate vial that contained 50 mg silanized glass wool. The vial was covered with aluminum foil, which prevented exposure of nonanal to sunlight. Nonanal, in 100 μL paraffin oil, was loaded onto the glass wool and the vial was capped. A microcapillary glass (31.75 mm long, 0.91 mm outer diameter, 0.43 mm i.d., 5 μL internal volume) that pierced through the cap septum allowed nonanal to evaporate from the vial. A metal paper clip held the nonanal dispenser and pheromone septum together and both were held by a steel alligator clip installed inside a Hartstack trap. The base of each trap was positioned just above the crop canopy on a steel rebar rod (1.5-m long) that was driven into the ground. Seven traps per treatment were examined daily over 7 days in a cotton plot located in the Central Crops Research Station at Clayton, NC (35° 40' 16.0" N, 78° 30' 35.9" W) in August 2019. Plants were between the flowering stage and formation of ripening bolls. Each block consisted of seven traps spaced 15 m apart. The second block was set 15 m away in a parallel row. Each treatment was replicated once within each block and repositioned daily to a new random trap position ($n = 9$). Trapped male FAW moths and all other moths were counted daily, and traps were rotated to new positions. All of the trapped moths were identified based on morphological characteristics. We tested the attraction of male FAW to (i) a control treatment (clean hexane

Table 1. Amounts and percentages of Z9-14:OAc, Z7-12:OAc and nonanal in 10 μL hexane of each mix tested in the olfactometer assays (Fig. 2)

	Pheromone component (ng)		
Treatment[a]	Z9-14:OAc	Z7-12:OAc	Nonanal
Control	0	0	0
Pheromone	100	0.58	0
Pheromone +0.05% nonanal	100	0.58	0.05
Pheromone +0.1% nonanal	100	0.58	0.1
Pheromone +1% nonanal	100	0.58	1.0

[a] The % nonanal is relative to the amount of Z9-14:OAc.

Table 2. Amounts and percentages of Z9-14:OAc and Z7-12:OAc, which were loaded onto rubber septa, and nonanal, which was loaded in 100 µL paraffin oil (Fig. 3)

	Pheromone component (µg)		
Treatment[a]	Z9-14:OAc	Z7-12:OAc	Nonanal
Control	0	0	0
1% nonanal	0	0	10
4% nonanal	0	0	40
Pheromone	1000	5.8	0
Pheromone +1% nonanal	1000	5.8	10
Pheromone +2% nonanal	1000	5.8	20
Pheromone +4% nonanal	1000	5.8	40

[a] The % nonanal is relative to the amount of Z9-14:OAc.

and clean paraffin oil), (ii) 10 µg and (iii) 40 µg nonanal alone (clean hexane in the septum), (iv) the pheromone mix alone (Z9-14:OAc and Z7-12:OAc, and clean paraffin oil in the dispenser), (v) pheromone mix with 10 µg nonanal (1% relative to Z9-14:OAc), (vi) pheromone mix with 20 µg nonanal (2% relative to Z9-14:OAc), and (vii) pheromone mix with 40 µg nonanal (4% relative to Z9-14:OAc).

We tested the effect of nonanal in commercial FAW pheromone lures in a sorghum crop at the Lake Wheeler Field Laboratory, Raleigh, NC (35° 43′ 31.7″ N, 78° 40′ 33.3″ W), and in cotton at the Central Crops Research Station. Four blocks were set as described above, and each contained eight traps with different treatments. Lures were purchased from ChemTica (P061 white bubble cap dispenser; Heredia, Santo Domingo, Costa Rica), Scentry (grey rubber septum; Billings, MT, USA), and Trécé (red rubber septum; Adair, OK, USA). Lures from Scentry and Trécé were tested in September 2019, and ChemTica lures were tested in August 2021. Scentry and Trécé lures contain Z9-14:OAc, Z7-12:OAc, Z9-12:OAc and Z11-16:OAc. Nonanal was formulated in the same way as described above and the nonanal-containing vial and the commercial lure were held together with a straightened metal paper clip. This set was installed inside a Hartstack trap as described above. We tested the attraction of male FAW to each commercial lure alone with clean paraffin oil in the glass vial dispenser and each lure coupled with 20 µg nonanal in the vial dispenser.

We tested the effect of two trap designs on male FAW captures, a Hartstack trap and a Bucket trap, with the same commercial pheromone lure. The Bucket trap, also known as a Universal moth trap (GL/IP-2352; Yellow/White; distributed by Great Lakes IPM, Vestaburg, MI, USA), consists of a white container (bucket) and a yellow funnel lid with a green top. The pheromone lure was placed in a green-colored basket within the green top. A Hercon Vaportape II DDVP insecticidal strip (2.54 × 10.2 cm, GL/HC-8001-01; distributed by Great Lakes IPM) was placed inside the bucket to kill the trapped moths. The experiments were conducted in a cotton crop at the Central Crops Research Station. ChemTica lures were used in both traps without nonanal. Each block consisted of two traps that were 30 m apart. The second block was set as a parallel row 30 m away from the first block. Each treatment was replicated once within each block and rotated between the two trap positions ($n = 4$).

Because the Bucket trap was found to be more effective than the Hartstack trap, we repeated the experiments with nonanal and commercial lures in a sorghum crop (Lake Wheeler Field Laboratory) and cotton crop (Central Crops Research Station). Two blocks with ChemTica lures containing four different randomly assigned treatments and four blocks with Scentry and Trécé lures, each containing eight different randomly assigned treatments, were set up as described above. The pheromone lure was placed in the green basket within the green top and nonanal was dispensed from 3.5 × 3.5 cm polyethylene sachets (0.102-mm thickness; Uline, Pleasant Prairie, WI, USA). Nonanal dispensers were prepared by dissolving 20 µg nonanal in 100 µL hexane, which corresponded to 1% of the amount of Z9-14:OAc, loaded and heat-sealed in a polyethylene sachet and placed in the green basket together with the pheromone lure. We tested the attraction of male FAW to each commercial lure coupled with 20 µg nonanal in 100 µL hexane in the polyethylene sachet, and each lure coupled with 100 µL hexane in the sachet dispenser (control).

2.7 Statistical analysis

All statistical analyses were performed in R (v4.2.0) with α set to 0.05. Behavioral responses of male FAW to various blends in the olfactometer were analyzed by a binomial generalized linear model (GLM). Numbers of male FAW captured in field experiments were analyzed by a Poisson GLM. Treatments that had no catches were not included in the analyses. Species-specificity of treatments was tested by calculating the proportion of nontarget noctuid moths of the total moths captured per trap. This value was transformed (arcsine-square root), and captures were compared between traps with pheromone only and traps with pheromone plus nonanal using a one-tailed Student's *t*-test.

3 RESULTS AND DISCUSSION

Attractant sex pheromones are excellent tools for guiding and implementing IPM programs because (i) males are highly mobile and respond to extremely low amounts of pheromone; (ii) pheromone-baited traps reliably predict when adults fly, and adults are much more susceptible to insecticide and biocide treatments than larval stages that bore into stems, leaves and fruit; (iii) sex pheromone dispensers can effectively suppress populations through 'mating disruption'; and (iv) pheromone-baited insecticides and biocides can be used in attract-and-kill campaigns. FAW pheromone lures are used globally, and their use in traps is recommended by international agencies, such as the Food and Agriculture Organization (FAO) of the United Nations.[15]

However, there is evidence that the attractiveness of FAW lures can be improved both quantitatively (more moths trapped) and qualitatively (fewer nontarget species attracted).[7,8,10,12] Here, we identified nonanal as a new FAW female sex pheromone component that significantly synergizes the attraction of FAW males in both laboratory and field experiments. We documented the effects of nonanal using different pheromone lures, two different types of traps and several crops in various locations.

3.1 Identification of bioactive compounds using the antenna as a detector

Electrophysiologically active sex pheromone gland compounds were identified using female pheromone gland extracts processed through GC-EAD, with male antennae as biological detectors of eluted compounds. In addition to the previously identified pheromone components,[5,7,9–12] we found a new bioactive compound that eluted at a much earlier retention time (10.2 min; Fig. 1) than the previously identified 12–16 carbon acetate esters and aldehydes. We identified this new compound by MS as nonanal and confirmed the identification with an authentic nonanal standard. The amount of nonanal was 3.0% relative to the major pheromone component, Z9-14:OAc. No compound in the clean hexane (control) elicited any EAD responses. Nonanal is an unusual sex pheromone component of moths, and to our knowledge, the only other noctuid moth species where nonanal was extracted from the female sex pheromone gland is the cotton bollworm *Helicoverpa armigera*.[16] Although nonanal elicited strong EAD responses in *H. armigera*, its addition to a standard two-component pheromone blend failed to elicit greater responses in a two-choice olfactometer, possibly because of its high loading in rubber septa (5.2% relative to the major component Z11-16:Ald).[16]

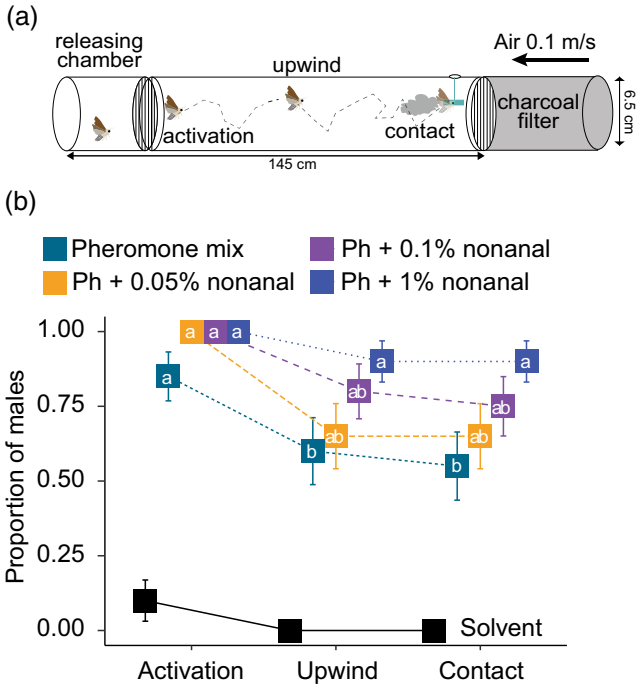

Figure 2. Behavioral responses of male FAW (*Spodoptera frugiperda*) to a synthetic pheromone mix with different doses of nonanal. (a) Schematic representation of the linear olfactometer. (b) Squares represent mean proportions of attracted moths ±SE ($n = 20$); the symbols representing the four treatments are offset so they do not overlap. Different letters denote significantly different responses within each behavioral response (binomial generalized linear model, $P < 0.05$). Solvent control was not included in the analysis as it only excited two males during the Activation step and none flew upwind.

3.2 Behavioral responses to nonanal

We conducted behavioral studies in a linear olfactometer to measure the effectiveness of nonanal as a component of the sex pheromone blend. The standard two-component pheromone blend of Z9-14:OAc and Z7-12:OAc activated 80% of the 20 tested males, but only 55% reached the pheromone source 110 cm upwind of the release point (Fig. 2). However, the addition of 0.05–1% nonanal increased the activation of males to 100%, and 65–90% of the males reached the pheromone source (Fig. 2), significantly more than with the two-component pheromone blend alone ($n = 20$, Binomial GLM, $P < 0.05$). The hexane control activated only 6.6% of the males, none of which reached the pheromone source. The olfactometer studies show that nonanal significantly synergizes the attraction of FAW males to the female sex pheromone.

3.3 Field tests with pheromone blends

We tested the effects of different doses of nonanal paired with a two-component pheromone blend consisting of 1000 µg Z9-14:

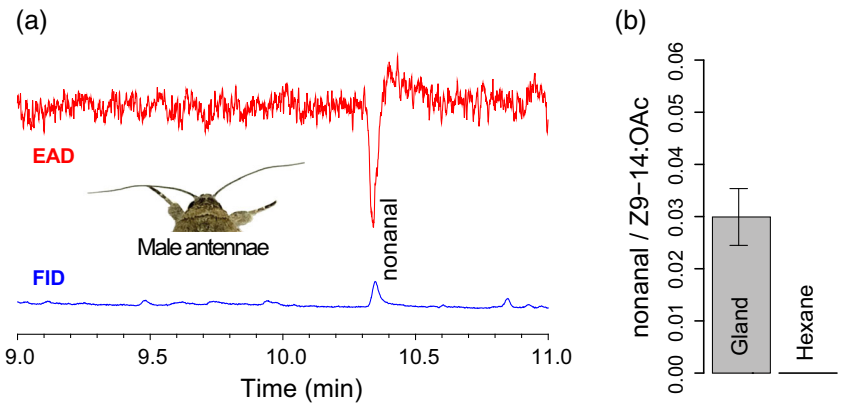

Figure 1. Nonanal, extracted from FAW (*Spodoptera frugiperda*) female sex pheromone glands, stimulates antennal responses in males. (a) Electrophysiological responses of a FAW male antenna to female sex pheromone gland extract (one female-equivalent, $n = 6$, but a single EAD trace is shown). (b) The mean ratio (±SE) of nonanal relative to Z9-14:OAc in sex pheromone gland extracts ($n = 6$). Hexane did not have any detectable Z9-14:OAc and nonanal.

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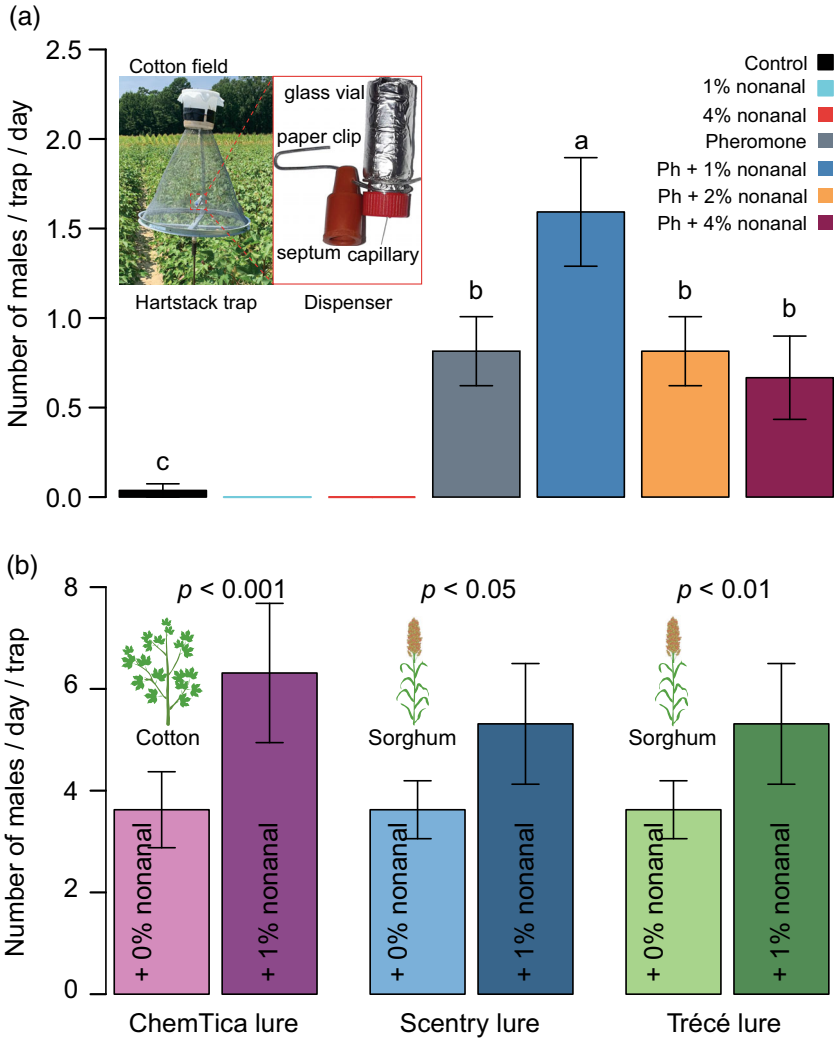

Figure 3. Field trapping of male FAW (*Spodoptera frugiperda*) using pheromone lures with and without nonanal. (a) Number of male FAW caught per day in Hartstack traps in a cotton field 15–20 August 2019. Nonanal was added relative to the amount of Z9-14:OAc (1 mg). Traps with nonanal alone (1% and 4%) did not catch any males. Traps with a two-component pheromone mix and 1% nonanal caught the highest number of males. Three traps per treatment were examined daily for 9 days. Bars represent mean catches (±SE). Different letters denote significantly different trap catches [Poisson generalized linear model (GLM), $P < 0.05$, $n = 9$]. (b) Number of male FAW caught per day in Hartstack traps with commercial pheromone lures and nonanal in a sorghum field in 2019 and 2021. Nonanal (20 µg) was tested in combination with each lure. Four traps per treatment were examined daily over 4 days ($n = 4$). Bars represent mean catches (±SE; Poisson GLM).

OAc and 5.8 µg Z7-12:OAc [Fig. 3(a) inset]. We used steel wire mesh traps (Hartstack) in a cotton field at the Central Crops Research Station. Nonanal was tested at three doses, 10, 20 and 40 µg, which corresponded to 1%, 2% and 4% of the amount of Z9-14:OAc, the major pheromone component. Traps baited with nonanal alone, at 1% and 4%, did not capture any males [Fig. 3(a)]. However, the addition of nonanal to the two-component blend significantly increased the numbers of male FAW in traps, with 1% nonanal doubling trap catch relative to the two-component pheromone mix alone [Fig. 3(a)]. Fewer males were trapped when higher doses of nonanal (2% and 4%) were deployed [Fig. 3(a)]. We captured a total of 121 moths in pheromone-baited traps, of which 105 were FAW males, one FAW female, and 15 other noctuid moths. Traps without nonanal captured 22 FAW males and five other noctuid moths (18.5%), whereas traps with nonanal (1%, 2% and 4% nonanal combined) captured 83 FAW males and 10 other noctuid moths (10.7%) (Table S1). Thus, as is typical of secondary components of moth sex pheromone blends, nonanal does not attract any males by itself, and is bioactive at a specific ratio relative to the major pheromone component. Moreover, despite the sparse captures of nontarget noctuid moths, there was marginally significant greater species-specificity (fewer nontarget moths) with the addition of 1% nonanal to the pheromone blend (Student's *t*-test on transformed proportions: $t = 1.660$, df = 22, $P = 0.0555$).

Next, we added nonanal to three different commercial FAW lures. We assumed that the amount of the major pheromone component, Z9-14:OAc, in the commercial lures was 2 mg. Because the addition of 1% nonanal relative to the major pheromone component yielded the highest trap catches in our previous experiments [Fig. 3(a)], we paired each commercial lure with a separate dispenser that contained 20 µg (1%) nonanal. The addition of nonanal significantly increased the number of FAW males caught per day compared to the commercial lures alone [Fig. 3(b)]. Overall, the addition of 1% nonanal increased male FAW trap catch by 74.1%, 46.6% and 53.1% with ChemTica, Scentry and

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Trécé lures, respectively. In all of these experiments, we captured 482 noctuid moths, of which 464 were FAW males, and only 18 were other noctuid species. Traps without nonanal captured 180 FAW males and 12 other noctuid moths (6.2%), whereas with nonanal we trapped 284 FAW males and six other noctuid moths (2.1%) (Table S2). Thus, fewer nontarget moths were captured with the addition of 1% nonanal to each of the three commercial lures, but these results were not statistically significant ($P = 0.086$, 0.156 and 0.134, respectively, for ChemTica, Scentry and Trécé lures). However, overall analysis revealed that the addition of nonanal to all three pheromone lures attracted significantly fewer nontarget moths, resulting in greater species-specificity (Student's *t*-test on transformed proportions: $t = 2.049$, df = 65, $P = 0.0223$).

We also compared the effectiveness of two trap types on FAW male captures using the ChemTica pheromone lure. We found a remarkable difference between the two trap types, with the Bucket traps catching eight-fold more male FAW than the Hartstack traps [Fig. 4(a)]. Overall, we captured a total of 760 moths in all of the traps, of which 755 were FAW males and five were other noctuid species. Of those, the Bucket traps captured 672 FAW males (89.0%) and Hartstack traps captured only 83 FAW males (11.0%) (Table S3). These results are in stark contrast to previous work on FAW, where Scentry *Heliothis* traps (functionally similar to the Hartstack trap) captured more FAW males than Bucket traps.[17]

We then tested whether nonanal increased the efficacy of the three commercial lures in Bucket traps, which are smaller and much easier to deploy than Hartstack traps. Consistent with the previous experiments with Hartstack traps, the addition of 1% nonanal to each pheromone lure significantly increased the number of males caught per day compared to the pheromone lures alone [Fig. 4(b)]. Overall, the addition of 1% nonanal increased trap catches of FAW males by 110.6%, 112.8% and 134.7% for ChemTica, Scentry and Trécé lures, respectively. In these experiments, we captured 1986 noctuid moths, of which 1963 were FAW males and only 23 nontarget noctuid moths; there were no FAW females trapped. Of these, traps without nonanal captured 625 FAW males and 12 other noctuid moths (1.9%), whereas with nonanal we trapped 1338 FAW males and only 11 other noctuid moths (0.8%) (Table S4). As with the Hartstack traps, fewer, albeit not statistically significant, nontarget moths were captured with each of the three commercial lures upon the addition of 1% nonanal ($P = 0.1745$, 0.359 and 0.037, respectively, for ChemTica, Scentry and Trécé lures), but an overall analysis revealed that pheromone lures with nonanal had greater species-specificity and attracted fewer nontarget moths (Student's *t*-test on transformed proportions: $t = 2.086$, df = 61, $P = 0.0206$). Thus, considering the trapping results with both types of traps, nonanal increases the species-specificity of FAW sex pheromone lures. However, more extensive field trials are needed in various locations with diverse noctuid fauna to extend these findings.

3.4 Implications and impact

In many moth species (Lepidoptera), females produce a blend of attractant sex pheromones of several chemical classes including monounsaturated alcohols, aldehydes, or acetates (the so-called Type I pheromones) and pheromones consisting saturated hydrocarbons, polyunsaturated hydrocarbons and their epoxide derivatives (Type II pheromones);[18] the FAW sex pheromone represents the former group. Type I pheromones are produced in specialized glands at the tip of the abdomen through desaturation and chain shortening (by 2-carbons) of even-carbon fatty acids, followed by the introduction of the respective terminal functional group (acetate for FAW), resulting in 10–18-carbon pheromone components. Thus, nonanal represents a departure from typical lepidopteran pheromone blends. It remains to be determined whether nonanal

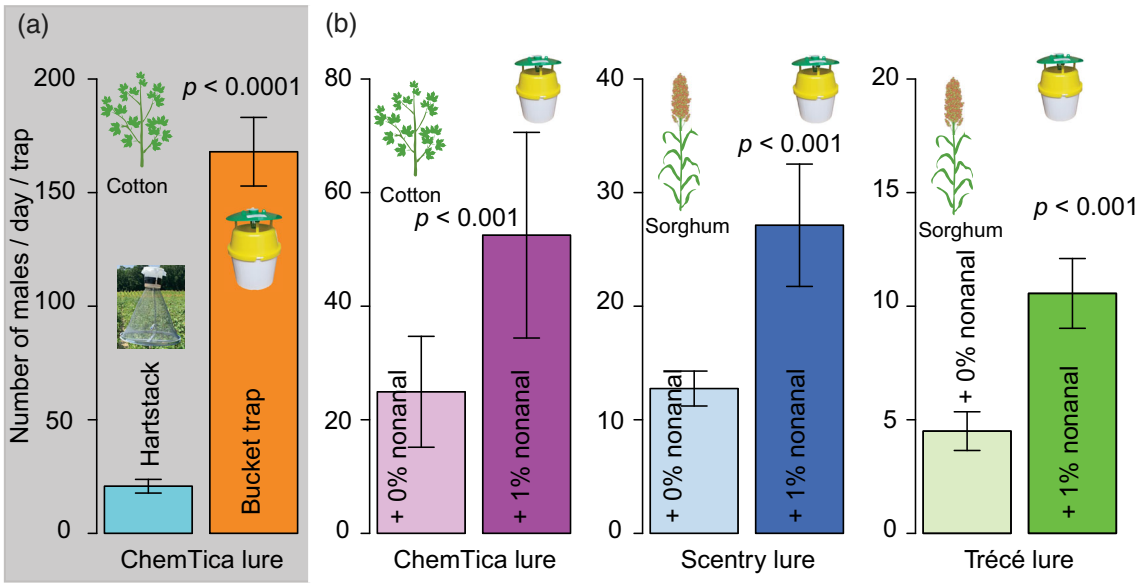

Figure 4. Number of male FAW (*Spodoptera frugiperda*) caught per day in two types of traps and the effects of adding nonanal to commercial pheromone lures in bucket traps. (a) Number of male FAW caught per day in bucket traps and Hartstack traps baited with ChemTica pheromone lure in a cotton field in 2021. Two traps per treatment were examined daily over 2 days ($n = 4$). Bars represent mean catches [±SE; Poisson generalized linear model (GLM)]. (b) Number of male FAW caught per day in bucket traps with various pheromone lures (ChemTica, Scentry, Trécé) without and with 1% (20 µg) nonanal in sorghum and cotton fields in 2021. The two blocks with ChemTica lures (two traps per treatment) were examined daily over 7 days ($n = 14$ per treatment) and the four blocks of Scentry and Trécé lures (four traps per treatment) were examined daily over 4 days ($n = 16$ per treatment). Bars represent mean catches (±SE; Poisson GLM).

is produced by female FAW, sequestered from plants, or represents a bioactive oxidation product of fatty acids or long-chain unsaturated hydrocarbons. Regardless, it serves an important function as a sex pheromone component.

Nonanal is a prevalent volatile compound emitted from flowers, nectar, leaves, fruits, microbes and various vertebrate animals, including humans, where it often serves as a semiochemical, attracting herbivores, parasitoids, ectoparasites and predators. For example, humans and birds emit nonanal, which attracts *Culex pipiens quinquefasciatus* mosquitoes and synergizes their attraction to CO_2.[19] As a putative pheromone component (involved in intraspecific communication), nonanal was found in some vertebrate species (e.g. ferret,[20]); see also Pherobase[21] for a more extensive list. As a sex pheromone component in insects, nonanal has been found in the wings of male milkweed butterflies[22] and male wax moths,[23] perhaps unsurprising because male sex pheromones often resemble plant-derived compounds. Within the Noctuidae, nonanal was recovered only from the pheromone glands of female cotton bollworm moths (*H. armigera*), but it did not appear to function as a sexual signal at the concentration tested.[16] Thus, our finding of nonanal as a sex pheromone component in FAW extends the range of moth attractant sex pheromones and suggests that the sex pheromones of other noctuid moth species should be re-examined for the presence and bioactivity of nonanal and related short-chain aldehydes.

Nonanal represents a particularly fascinating sex pheromone component because it is so widespread in nature as a plant-emitted volatile. Sex pheromones in moths are decoded through pheromone receptors and dedicated olfactory channels that project to a macroglomerular complex in the brain, separate from general odorants such as plant volatiles.[24] It will be intriguing and rewarding to uncover if nonanal stimulates pheromone receptors in FAW males, or whether it stimulates olfactory receptors, and its synergistic effect in mate finding results from interactions between ordinary glomeruli and the macroglomerulus within the antennal lobe.

Following its invasion and spectacular spread throughout Africa, Asia and Australia, the FAW has emerged as one of the most important global agricultural pests, threatening food security in many countries. It is imperative to develop new technologies to suppress surging FAW populations, and to optimize existing technologies. The addition of nonanal to the sex pheromone attractant blend promises to vastly improve the sensitivity of traps used in detection and monitoring of FAW and holds the potential to advance progress toward effective mating disruption of FAW populations. It is important to note that two host-associated FAW strains have been recognized. A corn-strain feeds preferentially on grasses such as corn and sorghum, and a rice-strain prefers small grasses such as rice, pasture grasses and millet.[25] The two strains differ in host preferences, physiology, mating behavior and pesticide susceptibility. Although geographic variation in their responses to pheromone blends has been noted,[26,27] males of both strains appear to respond to the same sex pheromone blend. Because the propagules of FAW that invaded Africa were the corn-strain from Florida,[28,29] the same strain used in our studies, we expect our findings to be applicable to invasive FAW populations in Africa, Asia and Australia.

4 CONCLUSION

The FAW, *S. frugiperda*, is a serious invasive polyphagous pest, currently considered the most destructive global pest of cultivated plants. This pest invaded the United States decades ago, and in the last 6 years has become established throughout Sub-Saharan Africa, Southeast Asia and China, the Pacific Islands and Australia, causing devastating crop losses. Through a series of analytical, electrophysiological, behavioral and field assays, we demonstrated that nonanal is a sex pheromone component of female FAW that significantly increased the attraction of conspecific male moths to a standard female pheromone mix that has been identified 37 years ago. Furthermore, we evaluated the efficacy of various pheromone blends, with and without nonanal. In all of our experiments, the addition of nonanal significantly increased the number of males trapped in cotton and sorghum fields whereas nonanal alone did not attract any male moths. Nonanal is inexpensive as a natural or synthetic compound and it is readily available globally. The addition of only 1% nonanal to existing pheromone blends can increase trap catch by 53–135%, which represents a large increase in the sensitivity of detection, monitoring and potentially mating disruption programs.

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CONFLICT OF INTEREST

A.M.S., E.H. and C.S. are listed as co-inventors on World patent WO2021178948A1, filed by North Carolina State University.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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