Trapping Soybean Looper (Lepidoptera: Noctuidae) in the Southeastern USA and Implications for Pheromone-Based Research and Management

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Trapping soybean looper (Lepidoptera: Noctuidae) in the southeastern USA and implications for pheromone-based research and management

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Abstract

Novel strategies for the management of soybean looper, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), are needed due to its increasing importance, costs, and challenges of management in soybeans (*Glycine max* [L.] Merr.; Fabaceae) in the Americas. Pheromone-mediated mating disruption may be 1 such novel strategy, but an effective tool for estimating mating disruption success must be found. The technique of trapping male moths using pheromone baited traps is a common method for assessing mating disruption technology. This paper reports on the testing of 2 trap types and 3 commercially available lures containing (Z)-7-dodecenyl acetate as the major active component. Trapping was conducted at 5 locations in the southern USA in the summer of 2019. Universal moth traps and delta traps were tested in combination with the Alpha Scents, Bio Pseudoplusia, and Scentry brand lures along with unbaited traps. Overall, both trap types were effective at capturing *C. includens* male moths, with universal traps having a higher capture rate. The Alpha Scents and Bio Pseudoplusia lures both had a significantly higher capture rate than the Scentry lure regardless of trap design. The active components of the Scentry lure differed from the others with the inclusion of 2 known minor pheromone components (esters) of *C. includens*, (Z)-7-dodecenyl propionate and (Z)-7-dodecenyl butyrate. The amount of (Z)-7-dodecenyl acetate of the Scentry lure also was lower than the Alpha Scents and Bio Pseudoplusia lures. The additional esters or unidentified components (impurities) of the Scentry lures may have influenced *C. includens* captures. A large number of *Ctenoplusia oxygramma* (Geyer) (Lepidoptera: Noctuidae) were captured in traps at 3 of the locations, though not in those traps baited with the ester-containing Scentry lure. Taken together, the data provide insight into effective trapping methods for *C. includens* and elucidates the need for researchers to understand the effectiveness of trapping components available in a region and the need for researchers to plan for captures of non-target species in pheromone-related research.

Key Words: *Chrysodeixis includens*; *Ctenoplusia oxygramma*; (Z)-7-dodecenyl acetate; (Z)-7-dodecenyl propionate; (Z)-7-dodecenyl butyrate

Resumen

Se necesitan estrategias novedosas para el manejo del medidor de la soja, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), debido a su creciente importancia, costos y desafíos en el manejo de la soja (*Glycine max* [L.] Merr.; Fabaceae) en las Américas. La interrupción del apareamiento por medio de feromonas puede ser una de estas estrategias novedosas, pero se debe encontrar una herramienta eficaz para estimar el éxito de la interrupción del apareamiento. La técnica de atrapar polillas macho utilizando trampas con cebo de feromonas es un método común para evaluar la tecnología de interrupción del apareamiento. Este artículo informa sobre las pruebas de 2 tipos de trampas y 3 señuelos disponibles comercialmente que contienen acetato de (Z)-7-dodecenilo como principal componente activo. Se realizó la captura en 5 ubicaciones en el sur de los EE. UU. en el verano del 2019. Se probaron trampas universales para polillas y trampas delta en combinación con los señuelos de las marcas Alpha Scents, Bio Pseudoplusia y Scentry junto con trampas sin cebo. En general, ambos tipos de trampas fueron eficaces en la captura de polillas machos de *C. includens*, con las trampas universales mostrando una tasa de captura más alta. Los señuelos Alpha Scents y Bio Pseudoplusia tuvieron una tasa de captura significativamente más alta que el señuelo Scentry independientemente del diseño de la trampa. Los componentes activos del señuelo Scentry diferían de los demás con la inclusión de 2 componentes de feromonas menores conocidos (ésteres) de *C. includens*, propionato de (Z)-7-dodecenilo y butirato de (Z)-7-dodecenilo. La cantidad de acetato de (Z)-7-dodecenilo del señuelo Scentry también fue menor que la de los señuelos Alpha Scents y Bio Pseudoplusia. Los ésteres adicionales o componentes no identificados (impurezas) de los señuelos Scentry pueden haber influido en las capturas de *C. includens*. También se capturó una gran cantidad de *Ctenoplusia oxygramma* (Geyer) (Lepidoptera: Noctuidae) en trampas en 3 de

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las ubicaciones, aunque no en las trampas cebadas con el señuelo Scentry que contiene éster. En conjunto, los datos brindan información sobre los métodos de captura efectivos para *C. includens* y aclarafica la necesidad de los investigadores para comprender la efectividad de los componentes de captura disponibles en la región e indica la necesidad para los investigadores de planificar las capturas de especies no objetivo en investigaciones de feromonas relacionadas.

Palabras Clave: *Chrysodeixis includens*; *Ctenoplusia oxygramma*; Acetato de (Z)-7-dodecenilo; Propionato de (Z)-7-dodecenilo; Butirato de (Z)-7-dodecenilo

The soybean looper, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) is a common pest of soybean, *Glycine max* (L.) Merr. (Fabaceae), and other crops in the Americas and Australia (CABI 2020). Larvae feed on soybean foliage and often cause sufficient defoliation to require insecticide application. Damage losses and management costs of *C. includens* in the USA were estimated to be $56.8 million in 2019, which included the majority of the range where *C. includens* is considered a pest in the USA (Musser et al. 2020). In Brazil, Argentina, Paraguay, and Uruguay, *C. includens* has increased its economic impact since 2000 (Bueno et al. 2017), and transgenic soybean expressing the *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (Bt) toxin Cry1Ac has been adopted commercially since the 2013/2014 crop season (Paula-Moraes et al. 2017). The Bt soybean technology has provided high levels of control (Bernardi et al. 2012). However, although this Bt soybean trait is considered a high-dose event in soybean, the risk of selecting resistant populations is still a challenge, especially due to the large area adopting this technology in South America (Brookes & Barfoot 2020). Additionally, in countries where the technology has not been released, management of *C. includens* with insecticides has become more difficult due to the development of insecticide resistant populations (Chiu & Bass 1979; Georghiou & Mellon 1983; Herzog 1988; Felland et al. 1990; Leonard et al. 1990; Boernel et al. 1992; Owen et al. 2013; Stacke et al. 2019, 2020). Therefore, novel approaches to its management are needed, especially with the increasing political and societal pressure to provide more ecologically sustainable pest management options due to climate change, population increases, public health, and environmental concerns.

Pheromone-mediated mating disruption of *C. includens* may be an option for ecologically sustainable management. Mating disruption uses synthetic pheromones to confuse male mate-finding behavior. Tumlinson et al. (1972) identified (Z)-7-dodecenyl acetate (Z7-12Ac) as a primary component of the *C. includens* sex pheromone used by females to call males, and Linn et al. (1987) added (Z)-7-dodecenyl propionate (Z7-12Prop) and (Z)-7-dodecenyl butyrate (Z7-12Buty) as minor but active components. Currently, there is no commercially available mating disruption product for *C. includens*, and there have been no published reports of the use of pheromone-mediated mating disruption of *C. includens*; however, Mitchell et al. (1975a) showed a 93% trap capture reduction of plusiine moths (*C. includens*, *Ctenoplusia* [*Trichoplusia*] *oxygramma* (Geyer), *Trichoplusia ni* [Hübner] [Lepidoptera: Noctuidae]) in plots treated with Z7-12Ac. The use of pheromone traps baited with Z7-12Ac to attract and capture male *C. includens* was documented over 30 yr ago (Linn et al. 1987), and there are now a few *C. includens* lure suppliers in the Americas. The successful use of pheromone-baited lures and evidence of trapping disruption suggest that mating disruption is a potential novel management tactic for *C. includens*.

One of the first steps in developing pheromone-mediated mating disruption is the assessment of available lures and traps. Mating disruption often is measured by proxy, via assessment of the reduction of male trap captures in pheromone-baited traps deployed within a field where a mating disruption formulation has been applied, as compared with captures in fields without pheromone (Mitchell & McLaughlin

1982; Filho et al. 2000; Kerns 2000; Burks et al. 2020). Pheromone trapping may be considered more consistent and labor-efficient than other methods for determining mating rates, such as egg monitoring or the use of tethered females. Therefore, an effective lure and trapping system is needed to make effective assessments of potential mating disruption technologies. Furthermore, an understanding of how species respond to partial and whole blends of pheromone, or how different geographic populations respond is important in mating disruption concepts. Linn et al. (1988) found a greater level of response of *C. includens* males to a 5-component pheromone blend than to a single component Z7-12Ac blend. There is evidence in some species that geographic sub-populations may respond differently to pheromone blends. Unbehend et al. (2014) reported finding different responses of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) populations to blends, doses, and a stereoisomer of its sex pheromone blend. Gao et al. (2020) found significant geographic variation of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) regarding female pheromone composition, pheromone blend effects on male capture, and antagonism of (Z)-11-hexadecenyl acetate. It is likely that commercially available lures may differ in their respective pheromone blends and, therefore, that these blends may differ in effectiveness across geographies. Therefore, lures should be tested to determine their effectiveness for unique geographies.

In addition to the need to understand the interaction of the target species with its pheromones, it is important also to consider the effects of the pheromone components on species within the subfamily Plusiinae, which has non-specific sex pheromones, and species isolation is primarily through behavioral and mechanical barriers (Eichlin & Cunningham 1978). If other taxonomically close species are very similar in appearance, are present in an area where a target species is being monitored with pheromone traps, and these species are attracted to the same pheromone lure components, then it is possible the cross attraction will compromise abundance estimation of *C. includens* and consequently lead to incorrect management decisions. In addition, cross-attraction can confound the results of trap capture data and confuse the outcome of mating disruption attempts. The primary pheromone component of *C. includens*, Z7-12Ac, has been recognized as a pheromone component in over 60 species globally, and as the primary component in 25 of these species (Pherobase 2020). It is reasonable to assume that in many geographies there are species that are taxonomically similar to *C. includens* and are attracted to Z7-12Ac, and therefore would be trapped in *C. includens* traps. For example, 3 Plusiinae species, *Trichoplusia ni*, *Ctenoplusia* (*Trichoplusia*) *oxygramma*, and *Rachiplusia ou* (Guenée) (Lepidoptera: Noctuidae) were collected in *C. includens* traps baited with "looplure" (Alford & Hammond 1982a) which is identified as containing only Z7-12Ac (NCBI 2021).

The trap being used also is important to consider and must be effective at capturing the target species. Successful trapping of *C. includens* has been documented with several types of traps, including electric grid traps (Tumlinson et al. 1972), pheromone-baited black light traps (Mitchell et al. 1975b), "wing" traps (Linn et al. 1987), Hartstack (wire cone) traps (Mink et al. 1993), universal moth traps (Meagher 2001), and delta traps (Zulin et al. 2018). These studies

indicated that *C. includens* can be trapped effectively using many commercially available trap configurations; however, some practical factors of trap type for the purpose of mating disruption monitoring must be considered. Traps used for mating disruption field-experiments and commercial-scale monitoring need to be cheap or reusable, create a minimal footprint in the agricultural landscape, and be maintained easily by commercial sales teams or growers. This limits the options to delta traps and universal moth traps. However, a further consideration is the number of moths a trap can capture. Although delta traps have been shown to be effective, are reusable (by having a replaceable sticky capture surface), and are relatively cheap, they are limited by the area of the sticky surface which can become less sticky as moth (or other insect) captures increase, or as dust and debris are blown into them. Universal moth traps share the same positive practical characteristics as delta traps but have a larger reservoir for holding captured moths, would be less influenced by wind-blown dust and debris, and decrease escape of captured moths when using killing strips.

In the present study, we evaluated commercially available trap and lure options for *C. includens*. The aim of the study was to determine if trap design or lure affect capture rates of *C. includens* and if the effect varies across locations. Additionally, we report the capture of other species.

Materials and Methods

EXPERIMENTAL LOCATIONS

Five trial sites were selected in the southern USA in 2019. Two trials were conducted in Louisiana, USA, 1 at the Louisiana State University Ag Center Central Research Station, Ben Hur, Louisiana, USA (LA-Ben Hur; 30.3687083°N, 91.1667527°W) and the other at the H. Rouse Caffey Rice Research Station, Crowley, Louisiana, USA (LA-Crowley; 30.2329806°N, 92.3783306°W). Two trials were conducted in Mississippi, USA, 1 at the Mississippi State University MAFES R.R. Foil Plant Science Research Center in Starkville, Mississippi, USA (MS-Starkville; 33.4777667°N, 88.7857917°W) and 1 at a commercial farm in Kiln, Mississippi, USA (MS-Kiln; 30.5076833°N, 89.3914278°W). One trial was conducted in Florida at a commercial peanut farm in Jay, Florida, USA (FL-Jay; 30.7770583°N; 87.1404000°W).

TRAPS AND LURES

Traps used in the trials were Scentry LP delta traps equipped with Scentry LPD liners (Scentry Biologicals, Inc., Billings, Montana, USA) and green bucket traps (often called universal moth traps or uni-traps; International Pheromone Systems, Ltd., Wallasey, United Kingdom) equipped with Hercon vaportape (Hercon Environmental, Emigsville, Pennsylvania, USA) as a killing agent. *Chrysodeixis includens* pheromone lures used in the trial were the Alpha Scents Soybean Looper lure (Alpha Scents, Inc., West Linn, Oregon, USA), the Scentry Soybean Looper lure (Scentry Biologicals, Inc., Billings, Montana, USA), and the Bio Pseudoplusia lure (Bio Controle, Indaiatuba, São Paulo, Brazil). Traps were placed on 1.4 m Trècè Pherocon Japanese beetle trap stands (Trècè, Inc., Adair, Oklahoma, USA) prior to the expected seasonal migration of *C. includens* to the areas. The LA-Ben Hur, LA-Crowley, and MS-Starkville trials were set up along the borders of soybeans on university research stations. The MS-Kiln trial was set up within a field of commercial soybeans. The FL-Jay trial was set up along the borders of a field of commercial peanuts. Traps were placed at least 30 m apart. Alpha Scents and Scentry lures were

used in traps at all 5 locations. Due to limited availability and delays of shipment arrivals, the Bio Pseudoplusia lures were used only at the FL-Jay, MS-Starkville, and LA-Ben Hur locations. Bio Pseudoplusia lures were used for the entirety of the FL-Jay trial, and were installed in the MS-Starkville trial on 31 Jul 2019 and in the LA-Ben Hur trial on 2 Aug 2019. Lures were replaced every 2 wk. Plastic gloves were worn and changed between traps to prevent cross-contamination of lures on traps. Scentry LPD liners were replaced at each evaluation or as needed. Hercon vaportape was placed in the universal traps and replaced monthly. In addition to lure baited traps, traps without lures were used. Each trap and lure combination was replicated 4 times and randomized at each location. Traps were monitored once or twice weekly depending on the location and moth populations. GPS locations of all traps were recorded.

MOTH IDENTIFICATION

Specimens collected at the LA-Ben Hur and LA-Crowley trials were identified using the key to moths commonly found in USA soybean fields (Herzog 1980). Identified species were compared with specimens in the Louisiana State Arthropod Museum, Baton Rouge, Louisiana, USA (https://www.lsuinsects.org/) to confirm identification. Specimens collected at the MS-Kiln and MS-Starkville locations were identified according to characteristics of wings including darker coloration of the forewings in comparison with hindwings and the presence of irregular silvery white spots on forewings (Carter & Gillett-Kaufman 2017). Identification was assisted by comparison to reference samples in the Mississippi State University Entomological Museum, Mississippi State, Mississippi, USA (https://mississippientomologicalmuseum.org. msstate.edu/) and by the Handbook of Soybean Insect Pests (Boernel & Higley 1994). In Florida, the identification of specimens of Plusiinae was performed initially by observing differences in marks and coloration on the forewings following Lafontaine and Poole (1991). Samples of specimens with similar wing pattern were submitted to male genitalia dissection adapting method from Pogue (2004) and used as reference material.

STATISTICAL ANALYSES

Prior to analyses, data were converted to the number of moths captured per night to account for irregularities between evaluation timings. To account for correlations due to proximity in space and time, moth counts were treated as Poisson random variables with the log Poisson rate being the sum of relevant Gaussian Processes across space (km) and time (d) (Neal 1998). There were 11 such Gaussian Processes, each with different mean and covariance matrices (1 for each treatment effect: 2 trap effects, 3 lure effects, and 6 interactions). Because trapping occurs over a period rather than a point in time, a discrete approximation was used: midpoints of the periods were used as the dates, and the Poisson rates were multiplied by the lengths of the periods. The squared exponential kernel was used in each case, with a separate scale parameter for each distance dimension. A full Bayesian approach was used to estimate the effects, as well as the Gaussian Process hyperparameters and latent values. Markov Chain Monte Carlo was performed in MATLAB (MATLAB, 2020. version 9.9 [R2020b], Mathworks, Natick, Massachusetts, USA) using a custom slice-within-Gibbs sampling algorithm (Geman & Geman 1984; Neal 2003) to draw 10,000 samples (40 chains of 250) from the posterior distribution after a burn-in of 2,750 and thinning by accepting 1 out of 100 for each chain; some serial correlation remained, rendering the effective sample size smaller than 10,000 independent samples.

Gas Chromatography/Mass Spectrometry/Flame Ionizing Detection

The active ingredient constituents of the lures were positively identified using coupled gas chromatography/mass spectrometry and were quantified with flame ionizing detection. Lures were extracted individually in vials containing 3 mL of 1 mg per mL C14FAME internal standards in hexanes. Samples were agitated with a vortex, and aliquots of the hexane layer were filtered into gas chromatography vials for analysis. Extracts were analyzed on a Hewlett Packard 6790 series gas chromatograph (Agilent Technologies, Santa Clara, California, USA) that was equipped with a capillary column (HP-IN-NOWAX, 30 m × 0.25 mm × 0.25 µm film; J&W Scientific, Folsom, California, USA) connected to an Agilent 5973N series mass selective detector (Agilent Technologies, Santa Clara, California, USA). The gas chromatography injection port was set to 240 °C. The column temperature was held at 125 °C for 3 min, then increased to 215 °C at 3 °C per min, and then to 240 °C at 25 °C per min, and held for 30 min. Helium (99.999%) was used as the carrier gas at 1.5 mL per min, and samples were injected at a ratio of 20:1 to prevent oversaturation of peaks. Samples also were analyzed using gas chromatography-flame ionizing detection equipped with an HP-88 column (60 m × 0.25 mm × 0.2 µm film; J&W Scientific, Folsom, California, USA). Samples were injected with a 20:1 split ratio at 240 °C, with helium as the carrier gas at 1.2 mL per min and a temperature program of a 100 °C hold for 1 min, then a 3 °C per min ramp to 240 °C, and a hold for 12.33 min. Pheromone active ingredients were tentatively identified by matches with the NIST 2005 mass spectral database (Kramida et al. 2020) before being confirmed.

Results

TRAPPING

There were a total of 11,278 *C. includens* moths captured and identified; populations varied between locations by the timing of peak and overall captures (Fig. 1). The timing of peak captures ranged from mid-Jul at the MS-Kiln location to mid-Sep at the FL-Jay location. Highest overall peak captures occurred at the FL-Jay location (about 10 moths per night per trap), and lowest overall peak captures occurred at the MS-Starkville location (about 2 moths per night per trap). Ranking of trap and lure combinations was relatively consistent across locations; therefore, data are reported as the average over all locations.

All trap and pheromone lure combinations caught significantly more moths than unbaited traps (Table 1). Alpha Scents, Bio Pseudoplusia, and Scentry lures all showed high likelihood of greater trap amplification in universal traps than in delta traps (99.9%, 97.8%, and 99.4% chance, respectively, where amplification was relative to non-baited traps) (Table 2). Thus, in both trap types, the Bio Pseudoplusia lure was more likely to have a higher daily trap capture rate than Alpha Scents. Additionally, there was high confidence of Scentry having the lowest daily trap capture rate relative to the other lures, regardless of trap type (Table 2). Universal traps had a higher daily trap capture rate than the delta traps for all 3 lure types (Tables 1, 2). The unbaited delta trap caught more moths than the unbaited universal trap (Tables 1, 2).

There were 1,294 non-target moths recorded across all locations, with the vast majority (832) identified as *C. oxygramma*, and they were recorded only from the Louisiana and Florida locations. Other non-target moths captured were *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (38), *S. frugiperda* (19), *Spodoptera ornithogalli* (Guenée) (Lepidoptera: Noctuidae) (89), *T. ni* (88), *Anticarsia gemmatalis* (Hüb-

ner) (Lepidoptera: Erebidae) (143), *Chloridea* (*Heliothis*) *virescens* (F.) (Lepidoptera: Noctuidae) (53), and *Hypena scabra* (F.) (Lepidoptera: Erebidae) (6).

Populations of *C. oxygramma* varied between locations by both the number captured and timings of peak captures (Fig. 2). The timing of peak trap captures ranged from mid-Jul at the LA-Ben Hur and LA-Crowley locations to mid-Sep at the FL-Jay location. Highest overall peak trap captures occurred at the FL-Jay location (about 5 moths per night per trap), and lowest overall peak captures occurred at the LA-Crowley location (about 0.4 moths per night per trap). Captures of *C. oxygramma* were sufficient to conduct analyses for differential response to the traps and *C. includens* lures. Because no *C. oxygramma* were recorded at the 2 Mississippi locations, these locations were not included in the analysis. Alpha Scents and Bio Pseudoplusia lures captured significantly more *C. oxygramma* than the unbaited in both trap types; however, Scentry lures did not (Table 3). The interaction of trap and lure was significant. The most attractive combination were the universal traps with the Bio Pseudoplusia lures followed by the universal traps with the Alpha Scents lures, with the others having very little probability (Tables 3, 4). Analysis of base capture rates between unbaited trap designs was inconclusive, because only 3 *C. oxygramma* moths were captured in unbaited traps.

GAS CHROMATOGRAPHY/MASS SPECTROMETRY/FLAME IONIZING DETECTION

All 3 lures contained Z7-12Ac as the primary pheromone constituent (Table 5; Supplemental Figs. 1, 2); the E-isomer also was identified in comparable quantities across all lures. The Scentry lure also contained 4.6% Z7-12Prop and 2.7% Z7-12Buty. The Alpha Scents lure contained a marginal 0.4% of the Z7-12Buty but no Z7-12Prop. Identification of the propionate and butyrate esters of the Scentry lure were validated with external standards (Supplemental Figs. 3, 4; Supplemental Table 1). Additionally, the Scentry lure had a large impurity (unidentified peak 2; Supplemental Fig. 5; Table 5), while the Bio Pseudoplusia lure had the least amount of impurities (Table 5).

Discussion

The number of *C. includens* moths captured in unbaited traps was low, but the base (without lure) capture rates were significantly higher for delta traps, indicating that accidental captures are more frequent for delta traps. The reason that any moths were captured in the unbaited traps is unclear. A possible explanation may be related to the sex of the moths that were captured. The majority of the moths in this study were not sexed, so it is plausible that some of those captured could have been females orienting to possible oviposition sites. The use of non-preferred oviposition sites has been documented in Lepidoptera (Jaumann & Snell-Rood 2017), and particularly in *S. frugiperda* (Gonçalves et al. 2020). Another explanation for capture in unbaited traps may be due to design differences, particularly the larger entrances into the delta trap. Moths that may be visually attracted to delta traps or incidentally fly through them can easily encounter the sticky pad and would not be able to escape. Moths encountering the universal traps would not be captured unless more directed orientation behaviors led them to fall through the funnel into the bucket. The white color of the delta traps versus the green color of the universal traps also may be more incidentally attractive due to its contrast against background colors. Although it is unclear if *C. includens* responds to trap color, *S. frugiperda* and *A. gemmatalis* have been shown to respond differentially to variations in the color
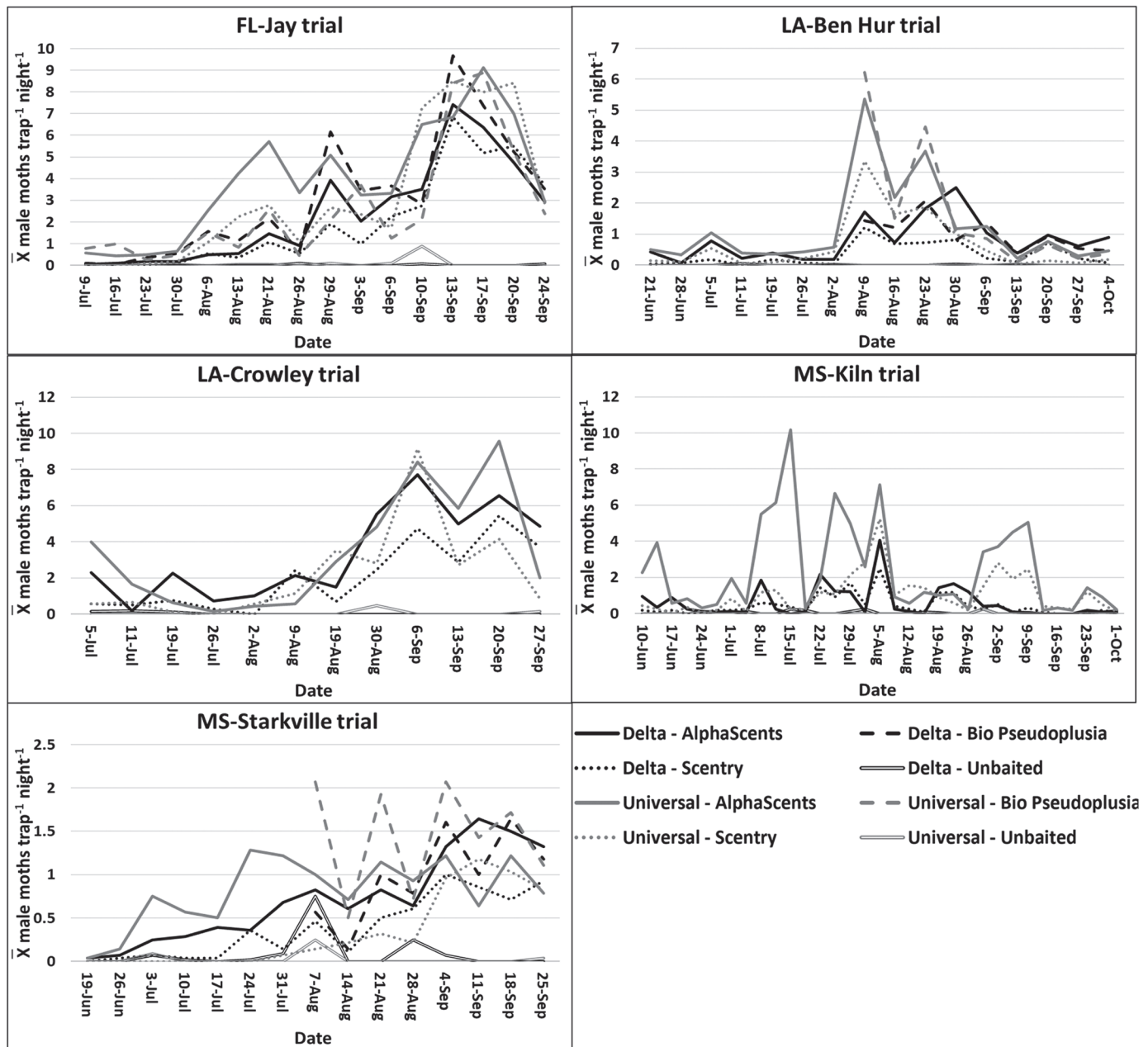
Fig. 1. Mean number of *Chrysodeixis includens* male moths captured at each of 5 trial locations. Note: Bio Pseudoplusia lures were not used at the LA-Crowley or MS-Kiln locations, were installed at the LA-Ben Hur location on 2 Aug 2019 and at the MS-Starkville location on 31 Jul 2019, and were installed at the FL-Jay location for the entire trial period.

scheme of universal moth traps (Mitchell et al. 1989). Although the proclivity for behaviors leading to incidental captures is unknown, it is important to understand base capture rates in pheromone-mediated mating disruption trials. If the mating disruption itself does not affect base capture rate, a trap with a high base capture rate would show a lower rate of disruption than a trap with a low capture rate and would therefore lead to artificially low assumptions of mating disruption. Trials with *C. includens* trapping, especially where mating disruption is being tested, should measure the base capture rate of moths and determine the sex of moths captured to help eliminate this area of uncertainty.

Both trap types appeared to perform well within the population densities that occurred, and although delta traps exhibited a higher

base trap capture rate (without lure), baited universal traps exhibited a higher trap capture rate than delta traps for all lures tested. Capture rates of *C. includens* using different types of traps has not been studied with pheromone lures. However, Whitfield et al. (2019) showed that universal and bucket type traps captured significantly more noctuids than delta or other sticky-type traps, and theorized that the reason for this is the difference in maximum capacity. Universal moth traps have large reservoirs that could potentially retain hundreds of moths, whereas the sticky surface of delta traps would become less effective at retaining captures as moths and debris accumulate, thereby reducing the sticky surface area. Regardless of the reason for higher capture rates with universal traps in this study, both trap types were consistently effective at capturing moths. However, the maximum capture

Table 1. Estimated daily trap capture rate (median and central 95% credible interval based on marginal posterior distribution) of *Chrysodeixis includens* captured in pheromone traps in 2019 across locations in Florida, Louisiana, and Mississippi.

Trap	Lure	N^a	Daily trap capture rate		
			2.5%	Median	97.5%
Delta	None	396	0.09	0.13	0.18
	Alpha Scents	368	1.41	2.75	4.96
	Bio Pseudoplusia	131	1.62	4.23	7.72
	Scentry	367	0.21	0.97	2.29
Universal	None	394	0.05	0.07	0.10
	Alpha Scents	363	2.67	4.58	6.73
	Bio Pseudoplusia	132	2.99	5.01	8.13
	Scentry	364	0.24	1.15	2.73

[a]The total number of trap and lure observations across all dates, locations, and replicates.

Table 3. Estimated daily trap capture rate (median and central 95% credible interval based on the marginal posterior distribution) of *Ctenoplusia oxygramma* captured in pheromone traps in 2019 across locations in Florida, Louisiana, and Mississippi.

Trap	Lure	N^a	Daily trap capture rate		
			2.5%	Median	97.5%
Delta	None	176	0.00	0.00	0.02
	Alpha Scents	176	0.06	0.14	0.29
	Bio Pseudoplusia	100	0.01	0.16	0.97
	Scentry	176	0.00	0.00	0.02
Universal	None	176	0.00	0.01	0.03
	Alpha Scents	176	0.19	0.65	1.73
	Bio Pseudoplusia	100	0.06	1.01	5.78
	Scentry	176	0.00	0.01	0.02

[a]The total number of trap and lure observations across all dates, locations, and replicates.

capacity of a trap design must be considered for locations where *C. includens* populations are larger or where there may be long periods between trap evaluations.

Our data indicated a strong influence of lure type on capture rate of *C. includens*. Bio Pseudoplusia and Alpha Scents lures had higher daily trap capture rates than Scentry lures, regardless of trap type. The lower purity of Z7-12 acetate and inclusion of the Z7-12 propionate and butyrate esters in Scentry may have contributed to this lower *C. includens* trap count. Therefore, it is possible also that the inclusion of the marginal 0.39% butyrate in the Alpha Scents lure, while not improving trap count, may not have been at a quantity large enough to create a significant decrease in attraction witnessed in the Scentry lures (though it was a marginally lower trap capture rate compared with the completely ester-free Bio Pseudoplusia lure). A negative effect from the esters is surprising, however, as they have been collected in the effluvia of calling females (Linn et al. 1987) and have been shown to increase attraction in a flight tunnel and some field trapping trials (Linn et al. 1988). Another explanation for the low trap captures of the Scentry lure may be unidentified peak 2. It may have an antagonistic effect on *C. includens*.

The total mass of each active ingredient was quantified in the lure extracts; however, no conclusions can be drawn on the overall release rate or active ingredient load of the lures. The extraction methods likely did not recover all active ingredients from the lures, or they may have extracted different amounts due to the physical design of the lures. Scentry and Alpha Scents were rubber septa, while Bio Pseudoplusia was a flat rubber disk.

Our study revealed *C. oxygramma* as a prevalent species cross-attracted during the trapping of *C. includens* in the Louisiana and Florida locations. This cross-attraction was expected because taxonomically close Plusiinae share the same main pheromone component, Z7-12Ac (Landolt & Heath 1986), *C. oxygramma* has been trapped in *C. includens* trapping trials in Louisiana (Alford & Hammond 1982b), and the use of Z7-12Ac for mating disruption has been shown to cause reduced trap captures of *C. oxygramma* (Mitchell et al. 1975a). The Bio Pseudoplusia lure had a slightly higher capture rate than the Alpha Scents lure, and both had significantly higher capture rates than Scentry. Similar to that previously discussed with *C. includens*, this likely may be due to differences in the components of the lures. The propionate and butyrate present in the Scentry lure and the marginal amount of butyrate in the Alpha Scents lure may serve as antagonists to *C. oxygramma*, contributing to better species specificity.

The frequent capture of *C. oxygramma* shows the importance of cross-attraction and non-target captures by pheromone traps. *Chrysodeixis includens* moths are difficult to distinguish from other Plusiinae moths that were present at the trial locations. Non-target captures that have superficial morphological similarities, e.g., *C. oxygramma* and *T. ni*, could be confused easily by untrained trap monitoring personnel, or due to moths losing some of the key superficial characteristics from degradation in traps between evaluations. When *C. includens* populations are high, the number of non-target captures may be of minor concern unless other morphologically similar species populations also increase, but in low *C. includens* populations, non-target captures that are incorrectly identified as *C. includens*

Table 2. Estimated probability of the combination of lure and trap (row) having higher daily trap capture rate of *Chrysodeixis includens* male moths compared to other combinations (columns) from trials across Florida, Louisiana, and Mississippi in 2019.

| Trap	Lure	Probability of higher daily trap capture rate vs.							
		Delta				Universal			
		None	Alpha Scents	Bio Pseudoplusia	Scentry	None	Alpha Scents	Bio Pseudoplusia	Scentry
Delta	None		0.00	0.00	0.01	1.00	0.00	0.00	0.01
	Alpha Scents	1.00		0.16	0.97	1.00	0.04	0.06	0.95
	Bio Pseudoplusia	1.00	0.84		0.98	1.00	0.42	0.32	0.98
	Scentry	0.99	0.03	0.02		1.00	0.00	0.00	0.23
Universal	None	0.00	0.00	0.00	0.00		0.00	0.00	0.00
	Alpha Scents	1.00	0.96	0.58	1.00	1.00		0.38	1.00
	Bio Pseudoplusia	1.00	0.94	0.68	1.00	1.00	0.62		1.00
	Scentry	0.99	0.05	0.02	0.77	1.00	0.00	0.00	
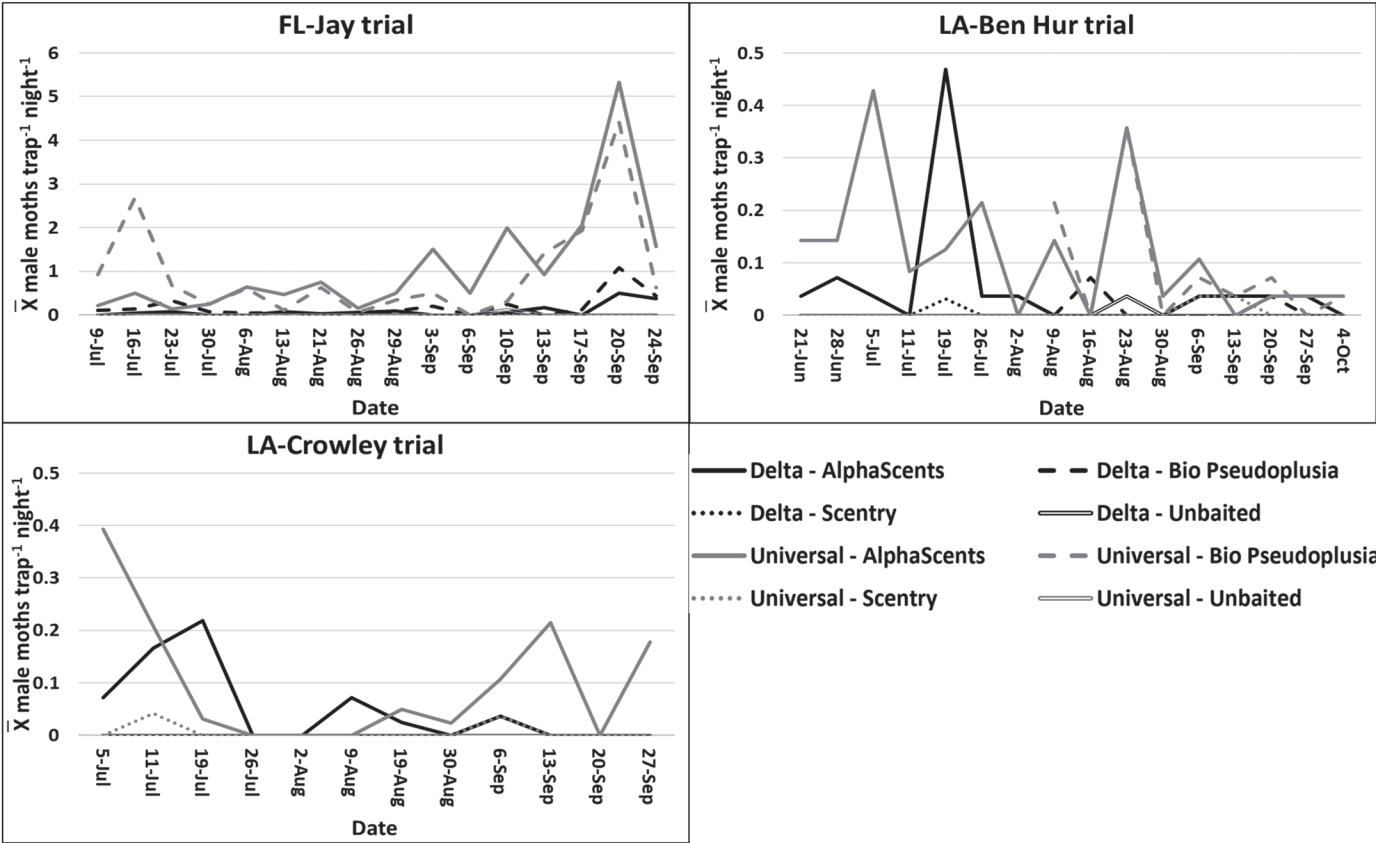
Fig. 2. Mean number of *Ctenoplusia oxygramma* male moths captured at each of 3 trial locations where they were recorded as present. Note: Bio Pseudoplusia lures were not used at the LA-Crowley location, were installed at the LA-Ben Hur location on 2 Aug 2019, and were installed at the FL-Jay location for the entire trial period.

could impact the results of trials significantly if there are relatively large numbers of misidentified, non-target species captured. The substantial inclusion of esters (together totally 9.4% relative to Z7-12Ac) in the Scentry product, while decreasing the overall catch of *C. includens*, may be more appropriate for environments where sizable non-target captures of *C. oxygramma* are possible. These results elucidate the need for researchers to be aware of the possibilities of other species being captured in traps, and ensure that the personnel counting moths are aware of this and take the appropriate steps to ensure the non-target species are accounted for in the data. This is especially relevant for the taxonomic group of Plusiinae.

The authors acknowledge that under ideal circumstances a second yr of data may have expanded results and highlighted any variations occurring across seasons that alter trapping efficiencies. However, due to the COVID-19 pandemic, the ability to conduct such research was in question at the time of 2020 trial planning. Similar trials were conducted later in 2020 in Brazil and other related-pheromone work is currently planned.

In summary, this study indicated that delta and universal traps are effective methods for capturing *C. includens* moths, and that the Alpha Scents and Bio Pseudoplusia lures are the most effective pheromone attractants available for *C. includens* moths in the southeastern USA. Addi-

Table 4. Estimated probability of the combination of lure and trap (row) having higher daily trap capture rate of *Ctenoplusia oxygramma* male moths compared to other combinations (columns) from trapping trials across Florida, Louisiana, and Mississippi in 2019.

		Probability of higher daily trap capture rate vs.							
		Delta				Universal			
Trap	Lure	None	Alpha Scents	Bio Pseudoplusia	Scentry	None	Alpha Scents	Bio Pseudoplusia	Scentry
Delta	None		0.00	0.02	0.69	0.17	0.00	0.00	0.49
	Alpha Scents	1.00		0.44	1.00	1.00	0.02	0.07	1.00
	Bio Pseudoplusia	0.98	0.56		0.99	0.96	0.09	0.00	0.98
	Scentry	0.31	0.00	0.01		0.11	0.00	0.00	0.31
Universal	None	0.83	0.00	0.04	0.89		0.00	0.00	0.81
	Alpha Scents	1.00	0.98	0.91	1.00	1.00		0.34	1.00
	Bio Pseudoplusia	1.00	0.93	1.00	1.00	1.00	0.66		1.00
	Scentry	0.51	0.00	0.02	0.69	0.19	0.00	0.00	

Table 5. Profiles of Scentry, Alpha Scents, and Bio Pseudoplusia lures used in the trapping trials.

Compound	RT (min.)	Scentry	Alpha Scents	Bio Pseudoplusia
Unidentified Peak 1	11.16		0.94%	
Unidentified Peak 2	12.05	15.04%		
Unidentified Peak 3	12.19			1.00%
Unidentified Peak 4	14.17	1.52%		
Unidentified Peak 5	16.86		0.45%	
12Ac	18.14	1.07%	0.31%	
Unidentified Peak 6	18.68		1.13%	
E7-12Ac	19.20	1.44%	2.43%	4.66%
Z7-12Ac	19.80	71.67%	87.20%	91.86%
Z9-12Ac	20.11	1.25%		
14FAME (IS)	20.95	N/A	N/A	N/A
Z7-12Prop	21.36	4.56%		
Unidentified Peak 7	21.66		0.40%	
Z7-12Buty	23.12	2.72%	0.39%	
Unidentified Peak 8	24.36			0.66%
Unidentified Peak 9	27.11			0.72%
Unidentified Peak 10	29.12		0.27%	
Unidentified Peak 11	31.72	0.72%	3.62%	
Unidentified Peak 12	32.63		0.38%	
Unidentified Peak 13	35.41		1.46%	
Unidentified Peak 14	39.26		0.31%	
Unidentified Peak 15	39.53		0.38%	
Unidentified Peak 16	39.78			0.52%
Unidentified Peak 17	40.70		0.33%	
Unidentified Peak 18	47.40			0.58%

tionally, *C. oxygramma* is a common non-target species that is captured in substantial numbers unless employing the Scentry lure. The results of these studies provide insight into important considerations for researchers conducting pheromone-based research and insect management.

Acknowledgments

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