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Seasonal Flight Patterns of *Chrysodeixis includens* (Lepidoptera: Noctuidae) in the Florida Panhandle and Inventory of Plusiine Species Cross-Attracted to Synthetic Pheromone

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Abstract

Chysodeixis includens (Walker) is a polyphagous economic pest in agricultural landscapes. To detect the occurrence of this pest in the field, trapping using sex pheromone lures is often implemented. However, other plusiine species are cross-attracted to these lures and may be misidentified as *C. includens* due to their morphological similarities. The objectives of this study were to provide region-specific information on the abundance of *C. includens* throughout the year as well as document the occurrence of related plusiines cross-attracted to *C. includens* sex pheromone traps in the Florida Panhandle. Twelve commercial fields of peanut and twelve commercial fields of cotton located across Escambia, Santa Rosa, and Jackson counties were monitored with Trécé delta traps baited with *C. includens* sex pheromone lures (Alpha Scents, Inc.; West Linn, OR) from June 2017 to June 2019. There was no difference in *C. includens* flight across dryland or irrigated fields. Identifications revealed that in addition to *C. includens*, the following species of the subfamily Plusiinae were crossed-attracted: *Argyrogramma verruca* (Fabricius) (Lepidoptera: Noctuidae), *Trichoplusia ni* (Hübner) (*Lepidoptera: Noctuidae*), Ctenoplusia oxygramma (Geyer) (Lepidoptera: Noctuidae), and *Rachiplusia ou* (Guenée) (Lepidoptera: Noctuidae). The occurrence of each species in the region and their flight phenology are documented. *Chysodeixis includens* abundance was greatest in September and decreased through December. Due to the high abundance of *C. oxygramma* and similar flight phenology to *C. includens*, this is the likeliest species to skew estimations and influence management decisions of *C. includens*, especially early in the crop season, when *C. includens* abundance is low.

Graphical Abstract
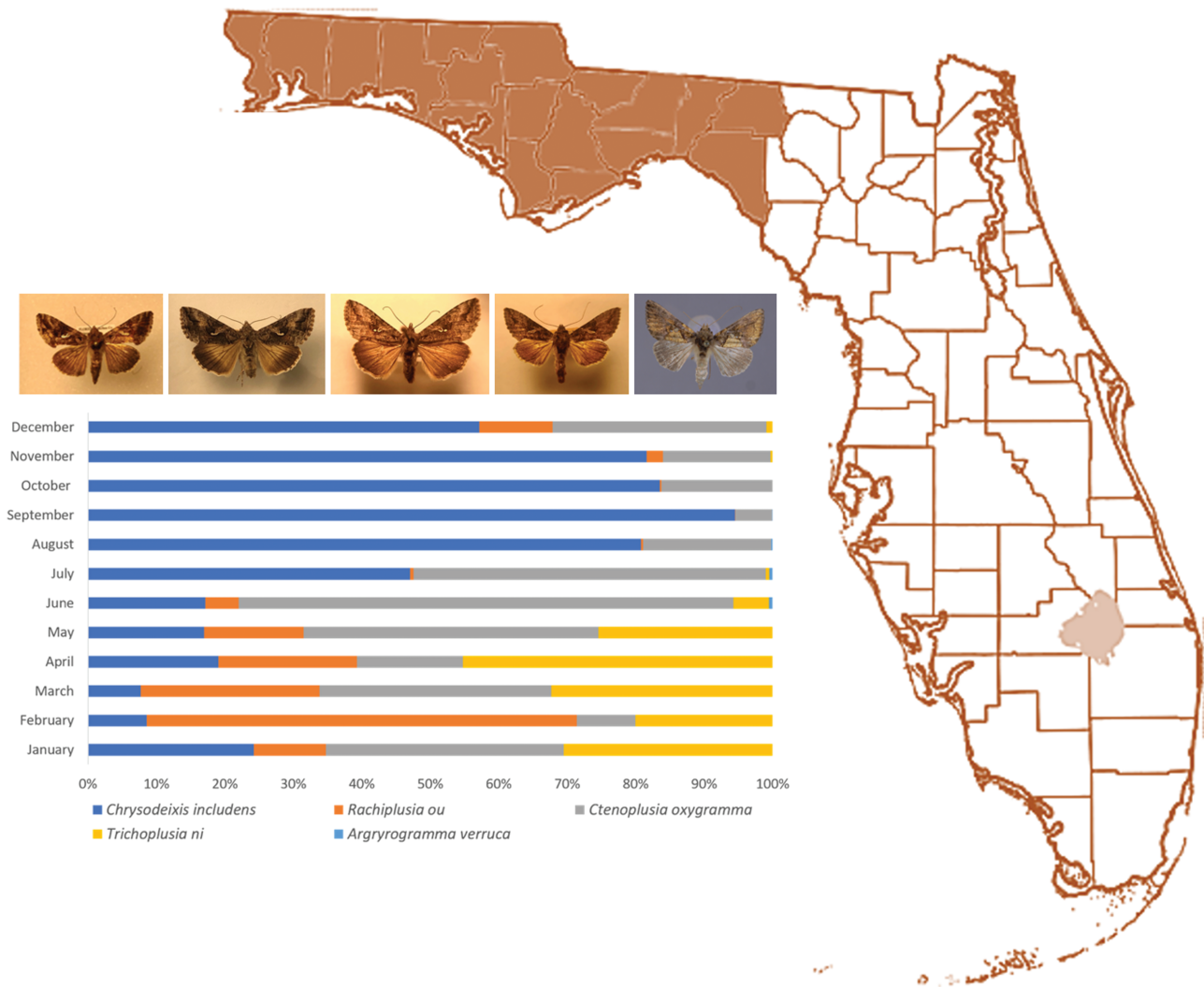
Key words: pheromone trap, population monitoring, cross-attraction, soybean looper

Soybean looper, *Chrysodeixis includens* (Walker) formerly *Pseudoplusia includens* (Walker), is a polyphagous pest of a variety of crops (Felland et al. 1992, Jost and Pitre 2002) with more than 174 species of host plants across 39 families (Specht et al. 2015). *Chrysodeixis includens* occurs in the United States as far north as Maine and south to Texas, but it is most abundant in the southeastern region of the United States (Herzog 1980). Its abundance in the southeastern United States (Herzog 1980) along with its polyphagous nature make *C. includens* a prevalent pest in the Florida Panhandle, which is an important region of peanut (*Arachis hypogaea*) (Linnaeus) and upland cotton (*Gossypium hirsutum*) (Linnaeus) production (USDA/NASS 2020). The Florida Panhandle is also an ecological transition zone between the subtropical and temperate climates through which populations migrate after overwintering as pupae in southern Florida (Mitchell et al. 1975, Smith et al. 1994). Monitoring the occurrence and abundance of *C. includens* in the region throughout the year is crucial in characterizing its population dynamics and determining timely implementation of management tactics (Pedigo and Rice 2009, Bueno et al. 2017).

Chrysodeixis includens belongs to the Plusiinae subfamily, which is widely distributed throughout the world, including several sympatric species in the southeastern United States (Mitchell et al. 1975, Eichlin and Cunningham 1978, Alford and Hammond 1982). Many of the plusiine species that occur in this region possess similar wing patterns in addition to the morphological traits shared across this subfamily (Eichlin and Cunningham 1978, Lafontaine and Poole 1991). Although these species are similar in appearance (Eichlin and Cunningham 1978), members within Plusiinae are diverse in their host range (Crumb 1956, Habeck and Tietz 1973, Eichlin and Cunningham 1978, Specht et al. 2015) and economic impact. While certain species such as *C. includens* and *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) are recognized as agricultural pests (Specht et al. 2019), many other species do not have a significant impact on agroecosystems, and for many others, an inventory of host plants remains incomplete (Eichlin and Cunningham 1978). Species within this subfamily have non-specific sex pheromones and are reproductively isolated through behavioral and mechanical barriers (Berger 1968, Eichlin and Cunningham 1978). Commercial formulations

of sex pheromone lures used to monitor *C. includens* are also non-specific ([Mitchell et al. 1975](#), [Alford and Hammond 1982](#), [Landolt and Heath 1987](#), [Linn et al. 1988](#), [Meagher 2001](#)), resulting in the cross-attraction of other plusiine species which may not be of economic concern. Thus, documentation of region-specific cross-attraction among species within this group is crucial when trapping populations with sexual pheromone formulations for ecological studies and pest management decisions.

The differences in the range of host plants across these plusiine species may translate to significant differences in economic importance. Therefore, the cross-attraction and presence of these species in trapping programs targeting *C. includens* could lead to false conclusions regarding pest population occurrence and size, pest risk, and management decisions. For instance, agriculture professionals unfamiliar with the minute phenotypical differences between plusiine species cross-attracted to *C. includens* commercial sex pheromones may overestimate the occurrence of this pest. Such misidentifications could result in the application of insecticides earlier or later in the season than necessary, especially if similar species exhibit disproportionate population densities throughout the year. The overestimation of certain pest densities could also lead to more frequent insecticide applications, creating unnecessary off-target effects in the environment ([Bueno et al. 2017](#)). While larval density in cotton and peanut ([Abney 2017](#), [Smith et al. 1994](#)) is largely used to inform farmers when to apply insecticides, the documentation of the timing, magnitude, and species cross-attracted during the pheromone trapping of *C. includens* represent a contribution to the adoption of timely management decisions. While there is no threshold for pheromone trapping of *C. includens*, trapping could provide an early-warning system for farmers, allowing predications for the occurrence of larval infestations after trapping of adults, and informing agricultural professionals when to start sampling pests in host crops. Therefore, a better understanding of the phenology of *C. includens* flight, as well as an inventory of plusiines cross-attracted to *C. includens* pheromone traps, will support region-specific management decision-making for the Florida Panhandle. In addition, this study may serve as a model

to other regions in the country regarding cross-attraction and identification of plusiine species for *C. includens* pheromone trapping programs.

The objectives of this study were to document the flight phenology of *C. includens* in the Florida Panhandle in commercial fields of peanut and cotton, and compare the effects of crop, irrigated, and dryland cropping systems, and trapping areas in the Florida Panhandle on *C. includens* abundance. In addition, the abundance of *C. includens* was compared across months. Furthermore, additional plusiine species which were cross-attracted to commercial *C. includens* sex pheromone traps in the region were identified and inventoried, with their abundance compared to that of *C. includens* for month, crop, county, and crop system tested for *C. includens*.

Materials and Methods

Commercial Fields Pheromone Trapping

Year-round pheromone trapping of *C. includens* was conducted in commercial fields in the Florida Panhandle region, from the spring of 2017 (June 2017) to the spring of 2019 (June 2019). Twelve commercial fields of peanut and twelve commercial fields of cotton, totaling 24 commercial fields located across Escambia, Santa Rosa, and Jackson counties were monitored with Trécé delta (Trécé Inc Pherocon VI trap, Adair, OK) traps baited with *C. includens* sex pheromone lures (Alpha Scents, Inc.; West Linn, OR). While a variety of *C. includens* sex pheromone lures are available on the market which may vary in their attractiveness to *C. includens* as well as cross-attracted plusiine species, previous publications have relied on *C. includens* female sex pheromones from Alpha Scents such as [Jones and Duckworth (2017)](#). The pheromone lures used in the present study were also based on common adoption practices in the region. Peanut and cotton fields in each county were selected based on the uniformity of the field size (average size of each field equaled approximately 25 ha) and the cropping system adopted (fields under dryland and irrigation) in each county. However, cropping system information was missing for some fields (Walker II and Jones; [Table 1](#)).

Table 1. Global positioning system (GPS) coordinates of traps in peanut and cotton fields for each farm in Escambia, Santa Rosa, and Jackson Counties. June 2017 to June 2019

Farm code	County	Trap 1 coordinates	Trap 2 coordinates
B I	Escambia	30.875667, −87.461028	30.879389, −87.463361
D I	Escambia	30.743417, −87.364222	30.743417, −87.364222
Hn I	Escambia	30.981917, −87.466083	30.981861, −87.469889
D II	Escambia	30.743417, −87.364222	30.741239, −87.348778
H II	Escambia	30.988500, −87.463889	30.988500, −87.463889
B II	Escambia	30.907444, −87.432889	30.911694, −87.433222
W I	Escambia	30.907141, −87.446996*	30.907141, −87.446996*
W II	Escambia	30.900456, −87.4190152*	30.90456, −87.4190152*
B I	Jackson	30.802808, −85.081067	30.803257, −85.080049
Ws I	Jackson	30.909782, −84.992168	30.611829, −84.991970
W II	Jackson	30.618208, −84.982342	30.617778, −84.981933
F I	Jackson	30.906339, −85.084087	30.905588, −85.082885
F II	Jackson	30.904257, −85.090149	30.903218, −85.090083
P	Jackson	30.869771, −85142915	30.869066, −85.140774
D R	Jackson	30.873129, −85.474890	30.873677, −85.478505
D S	Jackson	30.839048, −85.447540	30.841203, −85.445913
B II	Jackson	30.872493, −85.022154	30.869122, −85.018494
T	Santa Rosa	30.779472, −87.138639	30.911806, −87.434611
D	Santa Rosa	30.933444, −87.170306	30.935750, −87.158917
J	Santa Rosa	30.872839, −87.159952*	30.872839, −87.159952*

*Coordinates indicate general location and not exact coordinates of the trap.

Peanut and cotton fields were located across twenty farms, with four farms having one field of cotton and one field of peanut which were simultaneously sampled during the study. There were some cases in which data was only recovered from one trap per field. Escambia, Santa Rosa, and Jackson counties were selected for pheromone trapping since they have the highest acreage of cotton and peanut in the Florida Panhandle (USDA/NASS, 2020). Four peanut and four cotton fields were sampled in each county. Two fields of peanut and two fields of cotton were irrigated while the other two fields of each crop were dryland in each county. During the study (2017–2018 crop seasons), peanut-cotton crop rotation was adopted in the 24 commercial fields under study. In this way, each field cultivated with peanut was changed to cotton the following season, and each field cultivated with cotton was changed to peanut the following season allowing for the same farms and fields to be sampled throughout the study. Each field had two Delta traps equaling a total of 48 traps and the locations of the traps were not changed over the course of the study (Table 1). The two Delta traps in each field were positioned at least 100 m apart on the edges of the field (average size of the field was 25 ha). The Delta traps were mounted around one meter above the ground on aluminum poles. The pheromone lures were replaced every four weeks. The Delta trap sticky liners were replaced every two weeks and the samples were stored inside 32 × 23 cm Ziploc plastic bags (S. C. Johnson, Racine, WI), placed in Styrofoam coolers packed with ice, and transported to the Entomology Laboratory at the West Florida Research and Education Center (WFREC), Jay, FL. The sticky liners were then stored in a –20°C chest freezer until the samples could be assessed for the presence of *C. includens* and related plusiine species.

Plusiine Identification

The identification of the specimens of *C. includens* and crossed-attracted species in the pheromone trapping study was first made by observing differences in forewing markings and coloration. Differences in wing patterns were used to determine the presence of species that are easily differentiated by variations in wing patterns, following Lafontaine and Poole (1991). Plusiine species which could not be determined by wing pattern alone due to the similarities across species, as well as damage or obscuration caused by the glue on the sticky liners were dissected and their genitalia removed for further morphological comparisons. The dissection of each moth and the removal of male genitalia was adapted from Pogue (2004). The moth abdomen was removed with forceps and placed in a 10% KOH solution for a 24+ h period (Pogue 2004, Brambila 2009, Brown et al. 2015). The abdomen was then placed in a water bath with dish detergent added to break the water's surface tension. The genitalia were then removed from the abdomen with forceps, brushed clean using a small paintbrush, and placed under a dissecting microscope (Zeiss, Oberkochen, Germany). Morphological differences in male genitalia were observed to determine the presence of cross-attracted plusiine species. Genitalia dissections were also implemented to confirm species determinations made using wing patterns and other morphological characteristics (Eichlin and Cunningham 1978, Lafontaine and Poole 1991). Reference material was prepared, including photographic material of adults and male genitalia of each species of plusiine caught during the pheromone trapping period.

Data Analysis

The effects of crop, county, month, and species on moth counts per trap were analyzed using generalized linear mixed model (GLMM). We used a negative binomial error distribution to account for the count data, which contained many zeros, with overdispersion. The model included species, month, crop system, and location as fixed effects. The higher-level interactions were not included because certain interaction combinations did not occur in our data (e.g., there were many months where some moth species were not detected in a crop, which would not allow us to test a species × month × crop interaction term). Additionally, preliminary analysis of the data suggested these interactions were not significant. Due to the nested nature of our sampling scheme, the following nested random effects were included: farm and additional factors nested within-farm as split-plot factors, including crop system, year, month, and species. Due to singularity, the random effects were reduced to two random effects: farm and a single term nested within-farm to account for crop, crop system, year, month, and species combinations. The GLMM was implemented using the glmer.nb function in the lme4 package in R, which uses an unstructured covariance matrix to model the random effects but when factors are nested, levels within a factor will assume equal covariances (Bates et al. 2015). An analysis of deviance table was calculated using the ANOVA function in the car package (Fox and Weisberg 2019). Models fit in the glmmTMB package with more complex covariance structures (e.g., autoregressive or unstructured) had convergence issues, possibly due to some species having very low abundances in some months. Output from models with the simple (compound symmetry) and complex (autoregressive or unstructured) covariance structure were similar but given the convergence issues we proceeded with the simpler covariance structure that treats the repeated component as a split-plot experiment rather than a time-series. To assess model fit, the assumptions of the model were evaluated by examining the residuals simulated from the DHARMa package (Hartig 2020). Additionally, we tested for autocorrelation in the residuals using the testTemporalAutocorrelation function in the DHARMa package, which did not show signs of residual autocorrelation (Durbin-Watson test on simulated residuals grouped by month: DW = 1.23, $P = 0.15$).

When we detected significant interaction terms, we used post hoc contrasts with Tukey adjustments to interpret the results of the interactions. To determine differences across months, post hoc mean contrasts were performed comparing each species to *C. includens*. We did not conduct comparisons among non-*C. includens* species because this was not a primary goal of our research. To determine differences in moth abundance due to differences in county geographic location, post hoc mean contrasts were performed for each species across Escambia, Jackson, and Santa Rosa counties. To determine differences in moth abundance due to differences in host plant suitability, post hoc mean contrasts were performed for each species across peanut and cotton. To determine differences in moth abundance due to differences in irrigation, post hoc mean contrasts were performed for each species across dryland and irrigated systems. To determine differences in moth abundance due to differences in plant growth phenology and flight phenology throughout the year, post hoc mean contrasts were performed for each species across all twelve months. These contrasts were constructed by pooling across all other factors using the emmeans package (Length 2020).

Results

The year-round trapping of *C. includens* in commercial fields in the Florida Panhandle indicated flights of plusiine species throughout the year (Fig. 1, Table 2). A total of 5,145 specimens of plusiine were caught across all sites over the course of the two-year study. The highest plusiine abundance, measured as the average number of
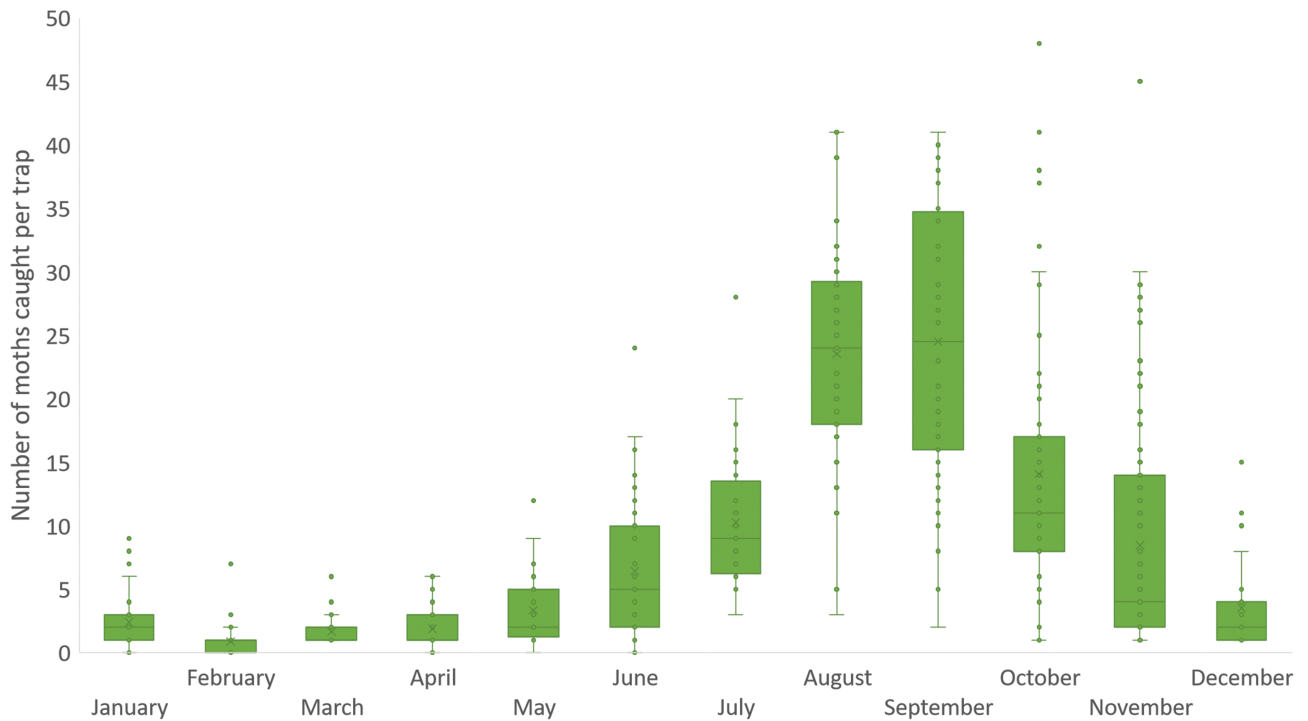
Fig. 1. Abundance of plusiines throughout the year using *Chrysodeixis includens* sex pheromone traps in commercial peanut and cotton fields of the Florida Panhandle (June 2017 to June 2019). The number of moths for each species across all years and all sampling sites were pooled according to month. The range within the bottom and top of each box indicate the 25th and 75th quartiles. The middle line within the box indicates the median value. The lines which extend outward from each side of the box show 1.5 times the interquartile range, while dots which fall outside these lines indicate outliers.

Table 2. Average monthly abundance of each species of plusiines detected per trap (± 1 SE), using *Chrysodeixis includens* sex pheromone in relation to the total of plusiines catch

Species of plusiines	Mean moth catch/trap											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Chrysodeixis includens	0.54 ± 0.15	0.07 ± 0.05	0.08 ± 0.05	0.31 ± 0.10	0.60 ± 0.16	1.11 ± 0.23	5.64 ± 1.18	28.57 ± 5.22	39.38 ± 6.83	15.49 ± 2.33	5.88 ± 0.97	2.13 ± 0.49
Ctenoplusia oxygramma	0.67 ± 0.17	0.09 ± 0.05	0.48 ± 0.15	0.23 ± 0.08	1.22 ± 0.27	4.09 ± 0.70	7.45 ± 1.51	6.10 ± 1.21	1.91 ± 0.43	2.17 ± 0.39	1.14 ± 0.22	0.98 ± 0.26
Rachiplusia ou	0.23 ± 0.09	0.51 ± 0.15	0.37 ± 0.13	0.34 ± 0.11	0.45 ± 0.13	0.38 ± 0.11	0.08 ± 0.06	0.11 ± 0.07	0.04 ± 0.04	0.02 ± 0.02	0.16 ± 0.05	0.12 ± 0.07
Trichoplusia ni	0.59 ± 0.17	0.16 ± 0.08	0.41 ± 0.13	0.72 ± 0.19	0.67 ± 0.18	0.26 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.03 ± 0.03
Argryrogramma verruca	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.02	0.07 ± 0.06	0.07 ± 0.06	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Contrast between cross attracted species and *C. includens*												
Ratio of *C. oxygramma*	ns	ns	ns	ns	ns	<0.001**	ns	5.0**	21**	7.0**	5.0**	ns
Ratio of *R. ou*	ns	ns	ns	ns	ns	3.0*	70.0**	250**	102**	810**	37**	17**
Ratio of *T. ni*	ns	ns	ns	ns	ns	4.0**	ns	NA	NA	NA	239**	62**
Ratio of *A. verruca*	NA	NA	NA	NA	NA	81**	79**	420**	4.04e9**	NA	NA	NA

Sampling performed in commercial peanut and cotton fields in the Florida Panhandle from June 2017 to June 2019. The lower part of the table contains magnitudes of change calculated from the estimated marginal means obtained from the main GLMM. Magnitude of change is calculated as (abundance of *C. includens*/other species), where a value of 1 would indicate equivalent abundances, values above 1 indicate *C. includens* is more abundant than the other species by X amount, and values below 1 indicate that *C. includens* is less abundant by X amount. Contrasts were conducted on the log-link scale of the negative binomial GLMM. Magnitude of change is only shown for significant contrasts: *$P < 0.05$, **$P < 0.01$ and ns = nonsignificant for the monthly mean ratio between the plusiine species and *C. includens*. NA indicates that the other species was not detected that month and no comparison was made.

moths caught per trap for the two-week duration each sticky liner was set in the field, occurred in August and September (Fig. 1) with an average of around 24 and 25 specimens caught per trap across all sites and years, respectively. After September, the population steadily decreased through December (Fig. 1) with an average of 14, 9, and 4 specimens caught per trap, for the two-week duration each sticky liner was set in the field, during October, November, and December, respectively. Identifications of plusiine species using wing

patterns and genitalia morphology revealed that cross-attraction to *C. includens* (Fig. 2) pheromone trapping occurs within four other plusiine species, including *Ctenoplusia oxygramma* (Geyer) (Lepidoptera: Noctuidae) (Fig. 2), *Rachiplusia ou* (Guenée) (Fig. 2), *Trichoplusia ni* (Hübner) (Fig. 2), and *Argyrogramma verruca* (Fabricius) (Lafontaine and Poole 1991) (Fig. 2). *C. includens* was the most abundant species trapped over the course of the two-year study (*n* = 3,667; 71.27%), followed by *C. oxygramma* (*n* = 1,166; 22.66%), *T. ni* (*n* = 161; 0.03%), *R. ou* (*n* = 144; 0.03%), and *A. verruca* (*n* = 7; 0.001%).

The effect of county (Escambia, Santa Rosa, Jackson), crop (peanut, cotton), crop system (dryland, irrigated), and month pheromone trapping occurred on total plusiine abundance was tested. There was an effect of month (χ^2 = 366.9, df =11, P < 0.001) and county (χ^2 = 10.4, df = 2, P = 0.006) on number of plusiines caught. There was not an effect of crop (χ^2 = 0.9, df = 1, P = 0.303) or crop system (χ^2 = 2.0, df = 1, P = 0.208). However, the five plusiine species differed in abundance (χ^2 = 365.5, df = 4, P < 0.001) and different patterns were detected for each of the five plusiine species when testing the interaction of species with county (χ^2 = 14.8, df = 8, P = 0.064), crop system (χ^2 = 22.1, df = 8, P = 0.005), and month (χ^2 = 546.0, df = 44, P < 0.001) of trapping.

Month was the only factor that affected *C. includens* abundance, as measured by the average number of moths caught per trap across all sampling sites and years. *C. includens* abundance was greatest in August and September with an average number of 28.57 and 39.38

specimens per trap, respectively (Table 2). These averages represent 81% of total plusiines caught during August and 95% of total plusiines during September (Fig. 3). The pheromone trapping indicated *C. includens* abundance as significantly higher than all other species in August and September. *C. includens* remained the most abundant species trapped from October through December (Fig. 4; Table 2), with an average number of moths caught per trap recorded at 15.49 for October (84% of total), 5.88 for November (82% of total), and 2.13 for December (57% of total; Figs. 3 and 4; Table 2). The average number of *C. includens* was significantly greater than all other plusiine species for these months (although note that some species had zero abundance in those months), with the exception of *C. oxygramma* in December, which did not differ from *C. includens* (*z* = 1.571, *P* = 0.516; Table 2).

County, crop, and month had a significant effect on the abundance of *C. oxygramma* (Table 3). The abundance of *C. oxygramma* was highest in Escambia county, with differences in means being significantly different across Escambia and Jackson counties (*z* = 2.801, *P* = 0.014; Table 3). *Ctenoplusia oxygramma* abundance was higher in cotton than in peanut (*z* = 2.468, *P* = 0.014; Table 3). *Ctenoplusia oxygramma also* had its highest average number of moths caught per trap in May through July, when *C. includens* abundance was relatively low (Fig. 4; Table 2). The average number of *C. oxygramma* caught during these months was 1.22 moths per trap were captured in May, 4.09 moths per trap in June, and 7.45 moths per trap in July (Table 2). The average number of *C. oxygramma* caught per trap
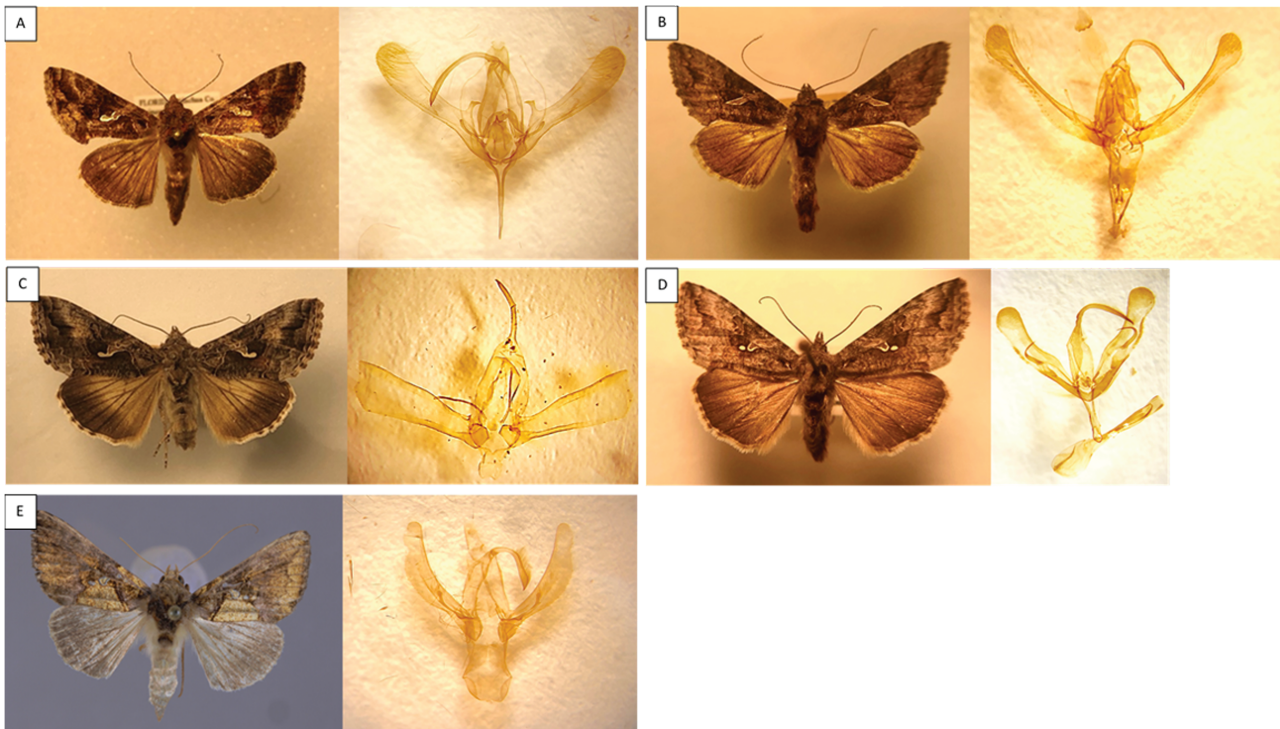

Fig. 2. (A) Adult specimen of *Chrysodeixis includens* (left) with the male genitalia (right). The genitalia possess a long, pointed saccus, long claspers, a hooked costal projection (Landolt) and valvae with a patch of setae at the terminal end. (B) Adult specimen of *Ctenoplusia oxygramma* (left)—this species is described as having a characteristic blade-like stigma. The male genitalia of *C. oxygramma* (right) possess spines long the valvae and three lobes on the terminal end of the saccus. (C) Adult specimen of *Rachiplusia ou* (left)—this species displays margined spots and stigma similar to *C. includens* and *T. ni* with similar variation. The male genitalia of *R. ou* (right) possess squared-off valvae and a short saccus with two lobes. (D) Adult specimen of *Trichoplusia ni* (right)—described as having a grizzled appearance and hind wings with a dark margin (Lafontaine and Poole 1991). This species displays reniform spots similar to *C. includens* and *R. ou* with similar variation. The male genitalia of *T. ni* (right) possess large recurved claspers. (E) Adult specimen of *Argyrogramma verruca* (right)—this species is described as the only plusiine species in the new world with golden forewings (Lafontaine and Poole 1991). The male genitalia of *A. verruca* (right) has a square shape of the saccus, whereas it is pointed in other genera of the Argyrogrammatini (Lafontaine and Poole 1991). This species also possesses an uncus with a row of setae.
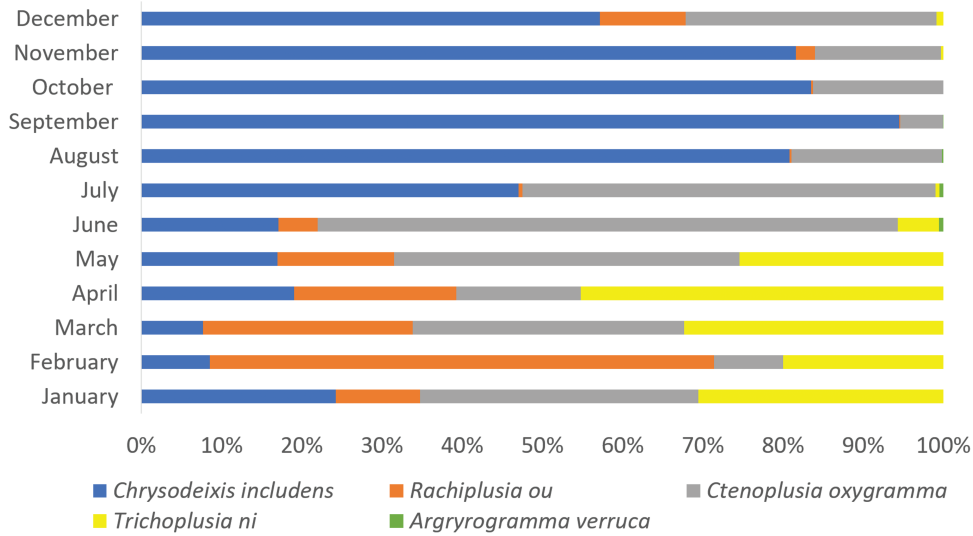
Fig. 3. Relative abundance of plusiines throughout the year using *Chrysodeixis includens* sex pheromone traps in commercial peanut and cotton fields of the Florida Panhandle (June 2017 to June 2019). The number of moths for each species across all years and all sampling sites were pooled according to month.
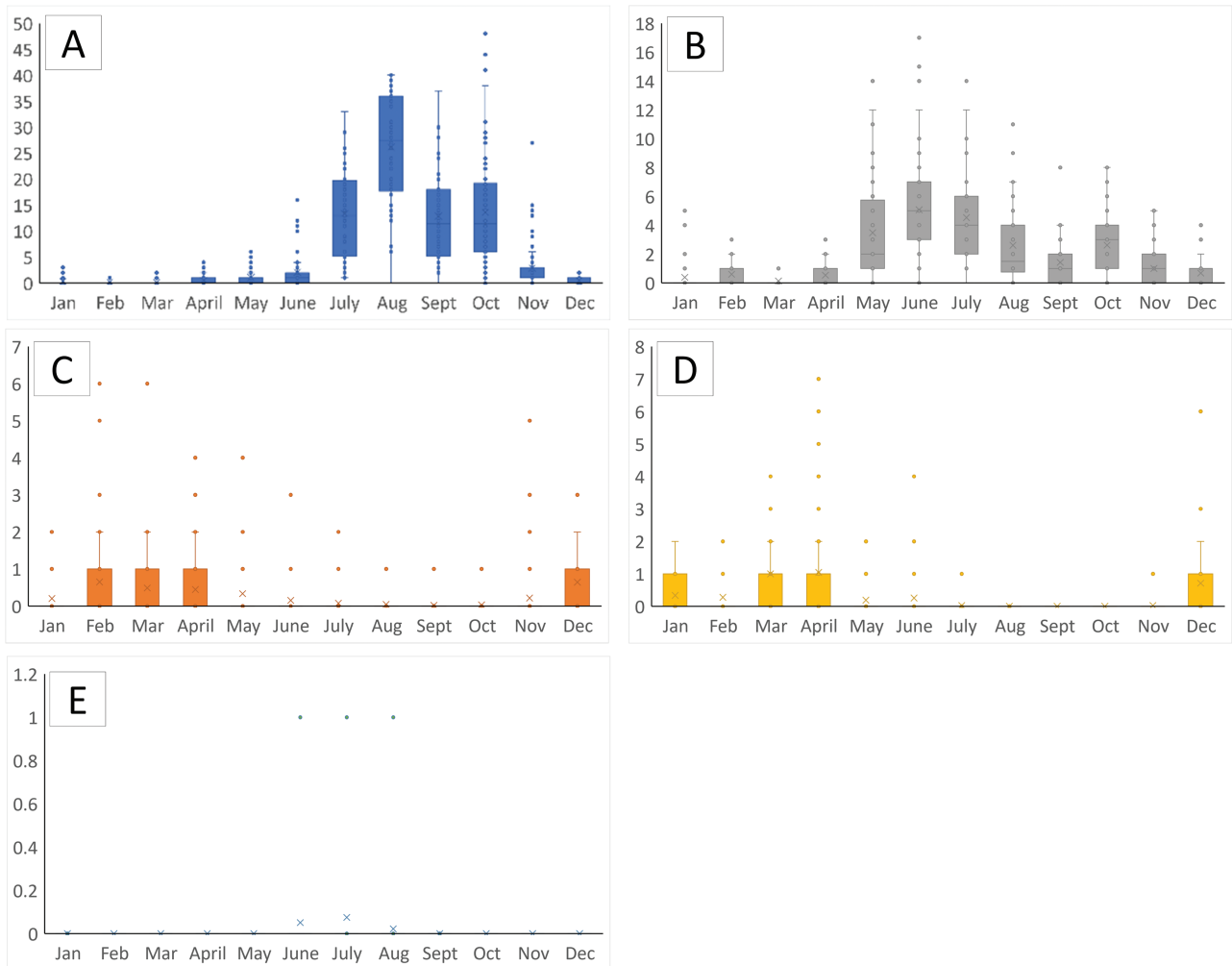

Fig. 4. (A) Abundance of *Chrysodeixis includens*, (B) *Ctenoplusia oxygramma*, (C) *Rachiplusia ou*, (D) *Trichoplusia ni*, and (E) *Argyrogramma verruca* caught throughout the year using *C. includens* sex pheromone traps in commercial peanut and cotton fields of the Florida Panhandle (June 2017 to June 2019). The number of moths across all years and all sampling sites were pooled according to month. Note that the scales on the *y*-axes differ among panels (A–E) to better show temporal changes in the abundance of each species. The range within the bottom and top of each box indicate the 25th and 75th quartiles. The middle line within the box indicates the median value. The lines which extend outward from each side of the box show 1.5 times the interquartile range, while dots which fall outside these lines indicate outliers.

Table 3. Effect of county, crop, crop system, season, month, and species on the abundance of plusiine moths captured in pheromone trapping in the Florida Panhandle from June 2017 to June 2019

		Results of contrasts among factor by species				
Variable	df	Chrysodeixis includens	Ctenoplusia oxygramma	Rachiplusia ou	Trichoplusia ni	Argryrogramma verruca
County	2	Escambia = 1.65 (0.25) A Jackson = 1.29 (0.21) A Santa Rosa = 2.03 (0.43) A	Escambia = 1.53 (0.22) A Jackson = 0.94 (0.15) B Santa Rosa = 1.20 (0.25) AB	Escambia = 0.30 (0.07) A Jackson = 0.09 (0.03) B Santa Rosa = 0.14 (0.05) AB	Escambia = <0.01 (0.17) A Jackson = <0.01 (0.14) A Santa Rosa = <0.01 (0.19) A	Escambia = <0.01 (0.00) A Jackson = <0.01 (0.00) A Santa Rosa = <0.01 (0.00) A
Crop	1	Cotton = 1.52 (0.21) A Peanut = 1.74 (0.26) A	Cotton = 1.39 (0.18) A Peanut = 1.03 (0.15) B	Cotton = 0.17 (0.04) A Peanut = 0.15 (0.04) A	Cotton = <0.01 (0.16) A Peanut = <0.01 (0.16) A	Cotton = <0.01 (0.00) A Peanut = <0.01 (0.00) A
Crop System	2	Dryland = 1.74 (0.21) A Irrigated = 2.04 (0.28) A	Dryland = 1.52 (0.31) A Irrigated = 1.02 (0.14) B	Dryland = 0.25 (0.05) A Irrigated = 0.13 (0.03) B	Dryland = <0.01 (0.14) A Irrigated = <0.01 (0.18) A	Dryland = <0.01 (0.00) A Irrigated = <0.01 (0.00) A

The column "variable" contains each factor in the model that was involved in an interaction with plusiine species. Each plusiine species is a column and levels within each species × variable combination are contained within the cells. Each cell contains the mean (±SE). Levels within a cell that differ based on linear contrasts ($P < 0.05$) are differentiated by having different capital letters.

was 43, 72, and 51% of the total number of plusiines for May, June, and July, respectively (Fig. 3). The abundance of *C. oxygramma* did not differ significantly from the target species, *C. includens,* in pheromone trapping during May or July (Fig. 4; Table 2). However, the abundance of *C. oxygramma* was significantly greater than the number of *C. includens* detected during the pheromone trapping in June (Fig. 4; Table 2). For August and September, when *C. includens* abundance was highest (Fig. 4; Table 2), the average number of *C. oxygramma* caught per trap was 6.10 for August and 1.91 for September, equaling approximately 19% of the average number of plusiines caught for August, and approximately 5% for September (Fig. 2; Table 2). *C. oxygramma* abundance was significantly different ($P < 0.05$) from all species during these months. For October, November, and December, when *C. includens* abundance decreased but remained the most abundant plusiine species (Fig. 4; Table 2), the average number of *C. oxygramma* caught was approximately 16, 16, and 31%, respectively (Fig. 3), and were significantly different than *C. includens* for October and November, but not December (Table 2).

The abundance of *R. ou* was significantly different across counties (Table 3), with a higher number of cross-attracted moths in *C. includens* pheromone traps occurring in Escambia than in Jackson county ($z = 4.198$, $P ≤ 0.001$). The abundance of *R. ou* was also significantly affected by crop system, with a higher number of cross-attracted moths in pheromone traps in dryland systems than irrigated systems ($z = 2.698$, $P = 0.007$; Table 3). The abundance of *R. ou* was also significantly affected by month with highest abundance of this species occurring early in the year (Table 2). The greatest *R. ou* abundance occurred in February through June (Fig. 4; Table 2). Of these months, the abundance of *R. ou* was around seven times greater than that of *C. includens* in February, and around 4.5 times greater than that of *C. includens* in March (Table 2). However, the abundance of *R. ou* was not significantly higher than *C. includens* in February or March (Table 2).

Overall, *T. ni* and *A. verruca* abundance was relatively low when compared with the other cross-attracted species in this study (Fig. 4; Table 2), with no effect of county, crop, or crop system on average number of moths caught per trap (Table 3). Month, however, did significantly affect *T. ni* abundance with the highest abundance of *T. ni* occurring in March with an average of 0.41 moths caught per trap (around five times as many *C. includens*), April with an average of 0.72 moths caught per trap (around two times as many *C. includens*), and May with an average of 0.67 moths caught per trap (about equal to the abundance of *C. includens*; Figs. 3 and 4; Table 2).

The abundance of *T. ni* when compared with those of *C. includens* during these months of high abundance indicates *C. includens* abundance did not differ significantly from *T. ni* in March, April, or May (Table 2). Seven specimens of *Argyrogramma verruca* were caught throughout the pheromone trapping with *C. includens* lure (Fig. 4) with no significant effect of crop, crop system, county, or month on *A. verruca* abundance (Table 3). Low abundance of *A. verruca* prevented accurate mean comparisons for most months, however, the abundance of *A. verruca* was significantly different from *C. includens* for June, July, August, and September (Table 2).

Discussion

The goal of population monitoring within an IPM framework is to prevent the implementation of control methods too early when the pest is not abundant enough in the crop to justify the costs of chemical spraying, or too late after the pest has reached economic damage levels (Pedigo et al. 1986, Pedigo and Rice 2009). Timely adoption of insecticides is often informed by population monitoring, (Pedigo et al. 1986, Cruz et al. 2010), which also poses other benefits, such as reducing environmental contamination and maximizing populations of beneficial arthropods (Higley and Pedigo 1993, Bueno et al. 2017), including natural enemies (Ramos et al. 2017, Pereira et al. 2018). Trapping adult lepidopterans is crucial for estimating the moth occurrence and abundance in the region which has broad implications for their control. One of the best methods of the timely estimation of noctuid adult populations and flight phenology is using traps baited with female sex pheromone lures (McNeil 1991), and there are many different traps that employ such pheromone lures (Whitfield et al. 2019). However, these trapping methods may contribute to misidentifications due to their means of capture as well as the lures used for these traps which cross-attract closely related species.

Two of the most common trap models used to capture noctuid moths in monitoring and population phenology studies are Delta and bucket traps (Whitfield et al. 2019). For this study, Delta traps were baited with a commercial formulation of *C. includens* female sex pheromone. There are many commercial formulations of *C. includens* sex pheromone lures, and potential differences across formulations could result in differences in strength of attraction exhibited by the different looper species reported in the present study. Estimating populations of pest species by trapping moths in Delta traps proved challenging due to the glue on the adhesive liners, which in some cases, obscured morphological features in the plusiine adults. This is especially true when moths attracted to the traps land

upside down on the adhesive liners. Bucket traps eliminate this problem posed by the adhesive liners. However, the destruction of wing patterns may still occur because the moths are permitted to fly around the inside of the container, causing the loss of wing scales due to repeated collisions with the sides of the container as well as other trapped moths. In addition, bucket traps present the issue of water accumulation from rain, as well as the issues of handling and storing the samples before they can be assessed. Overall, the use of adhesive liners allowed efficient storage of the samples after capture, as they were placed in transparent plastic bags and placed in a −20°C chest freezer before evaluations were performed.

The results of Plusiinae trapping throughout the year in this study adds information about the timing of *C. includens* flight in the southeastern United States. Previous reports have indicated high adult populations of *C. includens* September through October in central-northern Florida (Mitchell et al. 1975), and high populations in late August to September in corn, cotton, and soybean in Louisiana (Burleigh 1972). The present study is consistent with these results with the highest abundance of *C. includens* occurring in August and September and steadily decreasing through the end of the year (Fig. 4). In addition, the results of the present study are consistent with larval sampling in Mississippi, which illustrated the highest abundance of *C. includens* occurs in soybean from July through September (Allen et al. 2021). Thus, a clearer understanding of *C. includens* flight phenology may be valuable in predicting larval infestations in host crops, which has important implications for management decisions concerning this pest (Pedigo and Rice 2009), although most thresholds in the southern US are based on defoliation. For instance, the detection of initial moth flight could indicate the correct timing for larval sampling and management (Pedigo and Buntin 1994). Currently, in the case of IPM for *C. includens*, management decisions are largely based on larval abundance (Smith et al. 1994, Abney 2017). For example, the action threshold in peanut is four to eight larvae per crop row foot for chemical intervention to be considered (Abney 2017). However, monitoring adult abundance with pheromone traps has previously been shown challenging to predict larval populations of *C. includens* (Zulin et al. 2018) and needs further refinement for this species.

In addition to the timing of *C. includens* flight, the abundance of *C. includens* was tested for differences across counties, crops, and crop systems. *Chrysodeixis includens* abundance was not affected by these factors (Table 3). There was no difference in the abundance of *C. includens* across sampling sites, or between cotton and peanut, despite these two crops posing differences in their suitability as a host plant. One hundred percent of the cotton cultivated in the Florida Panhandle is transgenic, expressing insecticidal toxins of the bacterium, *Bacillus thuringiensis*, of which, *C. includens* remains a susceptible pest (Yano et al. 2015, Bel et al. 2017). In addition, there was no difference in *C. includens* flight across dryland or irrigated fields (Table 3). Thus, the similarities of *C. includens* abundance across these crops and crop systems, as well as across counties may be due to the capacity of *C. includens* to widely disperse in the agricultural landscape. However, a major complication in using pheromone traps to monitor *C. includens* abundance throughout the year is the non-specific nature of *C. includens* sex pheromone formulations which cross-attract sympatric species of plusiines (Mitchell et al. 1975, Alford and Hammond 1982, Landolt and Heath 1986, Linn et al 1988, Meagher 2001). If proper identifications are not made in population monitoring in ecological studies, the patterns observed and described in *C. includens* would not be as clear due to the wide differences in patterns of flight phenology and abundance across cross-attracted plusiine species.

During the screening and identification of specimens, a detailed inspection of wing patterns was performed. Specimens were included in the insect collection of the Entomology laboratory at the WFREC, and photographic documentation of adults and male genitalia of *C. includens*, *R. ou*, *C. oxygramma*, *A. verruca*, and *T. ni* specimens was prepared and included in the present study. This photographic documentation served as material for reference. Overall, the wing patterns of plusiines are both similar across species and variable within species, making this characteristic problematic for use in identifications of this taxonomic group. For example, *C. includens* is described as having strong brassy reflections, and a dark spot on the margin of the forewing (Lafontaine and Poole 1991). However, the brassy reflections described appear to be highly variable, and the dark spot on the margin is comparable to a similar trait in *R. ou*. *Trichoplusia ni* is described as having a hind wing that is darker towards the margin, as well as a grizzled appearance (Lafontaine and Poole 1991). This is further complicated by the fact that *R. ou* also tends to have hind wings with dark margins, as well as the description of "grizzled appearance" being an ambiguous characteristic. Furthermore, the three species described, as well as *A. verruca* all possess stigmas that vary greatly, sometimes joining, or existing separately, a variation that is also observed across these species. Despite having similar wing patterns, *A. verruca* possesses golden forewings that are unique to the Plusiinae of the New World (Lafontaine and Poole 1991). However, the golden coloration can be hard to determine whether the moth is upside down on the liners or has damaged wings.

For several specimens, the identification of plusiines demanded dissection and examination of differences in the male genitalia. Unlike wing patterns, the morphology of genitalia across Plusiinae, is diverse, allowing for accurate and reliable species identification. Thus, the use of Delta trap baited with *C. includens* sex pheromone lures paired with genitalia dissections of specimens proved to be a reliable method for moth capture and species identification in the present study.

The year-round flight phenology of *C. includens* in the Florida Panhandle has been documented. In addition to the contribution to IPM programs concerning *C. includens* flight and abundance, the present study presents an inventory of plusiine species cross-attracted to *C. includens* sex pheromone lures and the phenology of their flight in the region. Even though the detection of these plusiines was due to the cross-attraction of the commercial sex pheromone used to trap *C. includens*, rather than respective lures formulated for each cross-attracted species, the data in the present study represents a documentation of each species in the region, an estimation of their relative abundance, and the differences in their flight phenology compared to *C. includens*. While differences in the strength of attraction to *C. includens* sex pheromone lures across these species would result in differences in their rates of capture, and thus, not accurately reflect their true relative abundances in the landscape, their relative rates of capture in *C. includens* sex pheromone traps nevertheless can have wide implications for the management of *C. includens*. These differences in capture rates of each plusiine species throughout the year illustrate that the non-specific nature of pheromone traps used to estimate populations of *C. includens* could result in skewed estimations of the abundance of this target pest in the Florida Panhandle, which could lead to the unnecessary application of insecticides. This is especially true in the case of *C. oxygramma*.

Ctenoplusia oxygramma (Geyer) Lepidoptera: Noctuidae) is the only species belonging to the genus *Ctenoplusia* in North America (Lafontaine and Schmidt, 2010). This species occurs throughout the eastern and southwestern United States, as well

as occurring in the tropics (Eichlin and Cunningham 1978). This species is not described as being a pest of either cotton or peanut, though it is reported to be a pest of tobacco, and feeds on a variety of other non-commercial host plants (Crumb 1956). While *C. includens* remained the most abundant species in the late summer and early fall (Fig. 4; Table 2), *C. oxygramma* was the second-most captured species during this period (Fig. 4; Table 2). In addition, *C. oxygramma* was the most abundant plusiine species in May and June, prior to the period of high abundance of *C. includens* (Fig. 4; Table 2). The abundance of this species was also significantly higher in Escambia county and in cotton, which may be related to the presence of wild hosts prevalent in Escambia and in cotton fields (Crumb 1956). Its higher rate of capture during the early months of the cropping season (Fig. 4) and in cotton may indicate to Ag. professionals that *C. includens* abundance is unusually high in these situations if proper identifications are not made. In addition, the high rates of *C. oxygramma* capture during the peak of *C. includens* flight (Fig. 4; Table 2), may influence IPM decision-making for these months. However, unlike other plusiine species cross-attracted to *C. includens* pheromone lures, *C. oxygramma* has more characteristic wing patterns which easily differentiate this species from *C. includens* (Fig. 2), allowing for reliable identifications by agricultural professionals and thus, more timely application of insecticides.

In addition to *C. oxygramma*, among the plusiine species which are sympatric with *C. includens* in the Florida Panhandle (Eichlin and Cunningham 1978) and reportedly cross-attracted to *C. includens* sex pheromone lures (McLaughlin et al. 1975, Meagher 2001, Jones and Duckworth 2017), are *R. ou*, *T. ni*, and *A. verruca*. Each of these were detected in the present study. The plusiine species vary in their flight phenology (Eichlin and Cunningham 1978), and the range of host plants (Crumb 1956, Habeck and Tietz 1973, Eichlin and Cunningham 1978). However, their respective economic impacts have largely been unquantified. *Rachiplusia ou* has been reported to feed on a range of common plants, including commodities such as tobacco (Crumb 1956, Habeck and Tietz 1973, Eichlin and Cunningham 1978), contributing to its success in all areas in the United States with the exception of the Northwestern region since its invasion from South America (Eichlin and Cunningham 1978). *Rachiplusia ou*, was the third most abundantly caught species, and was caught in higher numbers than *C. includens* in the late winter and early spring (Fig. 4; Table 2) when peanut and cotton are not present in the landscape. Thus, the differences in its flight phenology compared to *C. includens*, along with its relatively low numbers makes this species unlikely to influence management decisions. *Argyrogramma verruca* is the only member of its genus in North America north of Mexico (Lafontaine and Poole 1991). It is widely distributed and is known to occur throughout the United States and Canada, occurring as far north as Ontario, south to Florida (Eichlin and Cunningham 1978), and West from Illinois to Colorado and Texas (Heppner 2003). This species is also known to occur every month of the year in Florida (Heppner 2003), though this species was only captured June through September in the present study (Fig. 4; Table 2). *Argyrogramma verruca* feeds on a variety of plants, including tobacco and cotton (Crumb 1956), though its economic impact on cotton is not quantified. While the number of *R. ou* and *A. verruca* specimens captured in *C. includens* pheromone traps was low (Fig. 4; Table 2), this may not accurately reflect the true abundance of this species if these species are only weakly attracted to *C. includens* sex pheromone lures. For this reason, further research on the economic impact of *A. verruca* in cotton would need to be conducted to determine whether its presence should be considered

in management decisions. *Trichoplusia ni* is widely distributed throughout the United States and Canada (Eichlin and Cunningham 1978). It is highly polyphagous, feeding on a wide variety of host plants including row crops such as tobacco and maize, as well as on citrus, ornamental plants, and weeds (Habeck and Tietz 1973, Eichlin and Cunningham 1978, Hoo et al. 1984). While the more suitable host plants of *T. ni* are the Brassicaceae (Specht et al. 2019), *T. ni* is also an economic pest of soybean. However, the economic losses caused by *T. ni* in the U.S. have not been quantified. *Trichoplusia ni* was captured in its highest numbers in January, April, and May (Fig. 4; Table 2) before the peanut and cotton cropping season, so this species is also unlikely to influence management decisions of *C. includens*.

Overall, the contribution of this study is to document the year-round flight and population dynamics of *C. includens*, as well as provide an inventory of the species of plusiines cross-attracted to *C. includens* trapping in the Florida Panhandle. In addition, the early flight of *C. oxygramma* during the crop season and its potential to skew estimations of *C. includens* has been indicated. This can lead to unnecessary adoption of chemical control, impacting the establishment of natural enemies and other beneficial arthropods early in the season in the region. *Ctenoplusia oxygramma* is not an economic pest in peanut and cotton and future Extension publications should include information about its flight in the region as well as the characteristics which can be used to identify this species. The distinctive presence of the blade-like stigma in the anterior wings of *C. oxygramma* is a reliable characteristic that agriculture professionals can use to differentiate this species from *C. includens* in field, when performing pheromone trapping of *C. includens*.

Acknowledgments

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