



**Attraction of A Native Florida Leafminer, *Phyllocnistis Insignis* (Lepidoptera: Gracillariidae), to Pheromone of an Invasive Citrus Leafminer, *P. Citrella*: Evidence for Mating Disruption of a Native Non-Target Species**

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# ATTRACTION OF A NATIVE FLORIDA LEAFMINER, *PHYLLOCNISTIS INSIGNIS* (LEPIDOPTERA: GRACILLARIIDAE), TO PHEROMONE OF AN INVASIVE CITRUS LEAFMINER, *P. CITRELLA*: EVIDENCE FOR MATING DISRUPTION OF A NATIVE NON-TARGET SPECIES

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## ABSTRACT

We collected a native North American species, *Phyllocnistis insignis* (Frey & Boll) (Lepidoptera: Gracillariidae), in traps baited with a 3:1 blend of (*Z,Z,E*)-7,11,13-hexadecatrienal (triene) and (*Z,Z*)-7,11-hexadecadienal (diene), 2 components of the sex pheromone of the invasive citrus leafminer, *P. citrella* Stainton. No moths were caught in unbaited traps during 6 months of monitoring. We evaluated seasonal abundance of *P. insignis* by monitoring traps in citrus (*Citrus* spp.; Sapindales: Rutaceae) groves at 4 sites in southeastern Florida during 2012. *Phyllocnistis insignis* moths were found in pheromone-baited traps year round with a peak flight in May. In trials designed to evaluate mating disruption of *P. citrella*, application of triene (SPLAT CLM<sup>TM</sup>) disrupted trap catch of *P. insignis* during a 9 week period following treatment in spring (825 mg triene/ha), but not winter (750 mg triene/ha). In a second experiment, application of triene (837 mg/ha) and a 3:1 blend of triene and diene (840 mg triene + 280 mg diene/ha, respectively) loaded onto rubber dispensers disrupted catch of male *P. insignis* during a 12 week period following treatment of 0.14 ha plots. Also, application of a 3:1 blend of triene and diene (764 mg + 253 mg/ha, respectively) formulated in SPLAT CLM disrupted trap catch of male *P. insignis* during a 4 week period following treatment in a 66 ha plot. In a third experiment, application of blend (837 mg triene + 278 mg diene/ha) reduced the incidence of trap catch to zero during a 16 week period following treatment of 0.87 ha plots. These data suggest that efforts to disrupt mating of *P. citrella* influence non-target populations of the congeneric leafminer species, *P. insignis*.

Key Words: citrus, congeneric species, leafminer, mating disruption, sex pheromone

## RESUMEN

Se obtuvieron especímenes de una especie nativa norteamericana, *Phyllocnistis insignis* (Frey y Boll), en trampas cebadas con una mezcla 3:1 de (*Z,Z,E*)-7,11,13-hexadecatrienal (trieno) y (*Z,Z*)-7,11-hexadecadienal (dieno), dos componentes de la feromona sexual de la minador de los cítricos invasiva, *P. citrella* Stainton. Ninguna polilla fue capturada en trampas sin cebo durante los seis meses de seguimiento. Se evaluó la abundancia estacional de *P. insignis* mediante el monitoreo de captura en trampas ubicadas en plantaciones de cítricos (*Citrus* spp.; Sapindales: Rutaceae) en cinco sitios en el sureste de Florida en 2012. Polillas de *P. insignis* se encontraron en trampas cebadas con feromonas durante todo el año con un vuelo máximo en mayo. En los ensayos diseñados para evaluar la confusión sexual de *P. citrella*, la aplicación del trieno (SPLAT CLM<sup>TM</sup>) redujo captura de *P. insignis* durante un período de 9 semanas después del tratamiento en la primavera (825 mg trieno/ha), pero no en el invierno (750 mg trieno/ha). En un segundo experimento, aplicación de trieno (837 mg/ha) y de una mezcla 3:1 de trieno y dieno (840 mg + 280 mg trieno dieno mg/ha, respectivamente) en dispensadores de goma redujo captura de machos de *P. insignis* durante un tratamiento de 12 semanas en parcelas de 0,14 ha. Además, la aplicación de una mezcla 3:1 de trieno y dieno (764 + 253 mg/ha, respectivamente) formulada en CLM SPLAT redujo captura de machos de *P. insignis* durante un periodo de 4 semanas después en una parcela de 66 ha. En un tercer ensayo, aplicación de una mezcla 3:1 de trieno:dieno (837 + 278 mg/ha, respectivamente) en dispensadores de goma redujo la captura de machos en trampas a cero durante un periodo de 16 semanas después del tratamiento en parcelas de 0,87 ha. Estos datos sugieren

que los esfuerzos para interrumpir el apareamiento de las poblaciones de *P. citrella* influyen poblaciones de *P. insignis*.

Palabras Clave: cítricos, especies congénéricas, minador de hojas, disrupción del apareamiento, feromona sexual

Citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is an invasive, multivoltine pest of citrus that became established in Florida in 1993 (Heppner & Fasulo 2010). The female sex pheromone of *P. citrella* is a 30:10:1 blend of (*Z,Z,E*)-7,11,13-hexadecatrienal (triene), (*Z,Z*)-7,11-hexadecadienal (diene), and (*Z*)-7-hexadecenal (monoene), respectively (Leal et al. 2006; Moreira et al. 2006). Male moths are attracted to lures (ISCAlure-Citrella™, ISCA Technologies, Riverside, California) containing a 3:1 blend of triene and diene (Lapointe et al. 2006). Applications of synthetic pheromones in citrus groves may be useful for controlling populations of *P. citrella* (Stelinski et al. 2008;

Lapointe et al. 2009; Stelinski et al. 2010). Trap catch is disrupted by applying either the blend or triene alone, and several substrates have been used to release these pheromone components, including rubber dispensers and a flowable wax matrix called SPLAT CLM™ (ISCA Technologies, Riverside, California) that can be applied using hand-applied dispensers or machines that propel dollops into the tree canopy (Lapointe & Stelinski 2011; Lapointe et al. 2011).

While trapping *P. citrella* adults in Florida citrus groves using pheromone lures optimized for this species (ISCAlure-Citrella), we observed consistent catch of a related native species, *Phyllocnistis insignis* (Frey & Boll) (Fig. 1A). This spe-



Fig. 1. Representative image of an adult male (A) *Phyllocnistis insignis*, a native North American leafminer of the Asteraceae and (B) *Phyllocnistis citrella*, the citrus leafminer, a highly invasive pest that originated in Asia, and became established in citrus growing areas of North Africa, North and South America and elsewhere during the 1990s.

cies is found only in the eastern United States from Florida to Michigan, and westward to Iowa (Minno 1992; Priest 2008; Anonymous 2012a; Durbin 2012). Its pheromone has not been identified, and little has been published about its natural history. Larvae create serpentine mines in leaves of host plants in the family Asteraceae, including the pale Indian plantain, *Arnoglossum atriplicifolium* (L.) H. Rob., the great Indian plantain, *Arnoglossum reniforme* (Hook.) H. Rob., American burnweed, *Erechtites hieraciifolia* (L.) Raf. Ex DC., golden ragwort, *Packera aurea* (L.) Á. Löve & D. Löve, butterweed, *Packera glabella* (Poir.) C. Jeffrey, and white rattlesnakeroot, *Prenanthes alba* L. (Busck 1900; Priest 2008; Eise-man & Charney 2010; Anonymous 2012b; De Prins & De Prins 2012). Of the known host plants of *P. insignis*, only American burnweed and butterweed grow in central and southeastern Florida, although no specimens of the latter species have been collected in St. Lucie County (USDA-NRCS 2012; Wunderlin & Hansen 2008).

If both species of *Phyllocnistis* are attracted to common pheromone components, then efforts to disrupt mating of *P. citrella* could also influence *P. insignis*. The objectives of this study were to: (1) verify attraction of *P. insignis* to pheromone lures deployed to attract *P. citrella*, (2) monitor phenology of *P. insignis* over an entire year, and (3) evaluate trap catch disruption of *P. insignis* males resulting from application of *P. citrella* pheromone components.

#### MATERIALS AND METHODS

The sites monitored and experiments performed were included as part of larger experiments to investigate mating disruption of *P. citrella*, during the course of which we also collected data on *P. insignis*. We caught moths in delta traps (Pherocon VI, Trécé, Adair, Oklahoma) and bucket traps (model 337-02, Trécé, Adair, Oklahoma). Each trap was baited with a pheromone lure (ISCALure-Citrella) that contained 1 mg triene and 0.33 mg diene. Except where otherwise noted, lures in traps were replaced approximately every 6-8 weeks. Delta traps were evaluated by counting moths caught within a 177 cm<sup>2</sup> area of sticky surface on liners changed every 1-2 weeks unless otherwise noted. Bucket traps were emptied into sealable plastic bags every 1-2 weeks, and male moths were counted in the laboratory. Each bucket contained a one-third piece (approximately 4 cm<sup>2</sup>) of Vaportape II killing strip (Hercon, Emigsville, Pennsylvania). We identified *P. insignis* moths by their characteristic pewter color and orange wing markings (Fig. 1A). On sticky liners, the moths were opaque and slightly larger than *P. citrella*, which appeared yellow with translucent wings. Voucher specimens of *P. insignis* were deposited at the Florida Museum

of Natural History McGuire Center for Lepidoptera and Diversity (Gainesville, Florida). The pattern of trap distribution differed among sites, but placement within trees was standardized. Traps were placed within citrus trees 1.5-2.0 m above the ground in the outer canopy, on the side of the tree facing the center of the bed. Traps were always separated by at least 50 m.

#### Lure Attraction and Longevity

Delta traps were used to monitor attraction of *P. insignis* to the pheromone of *P. citrella*, and unbaited traps served as a negative control for an experiment established during 2012 in a 28.4 ha block of mature 'Flame' grapefruit (*Citrus × paradisi* Macfad.) located in northwestern St. Lucie County, Florida (Site 1, N 27° 28' 18" W 80° 38' 13", Fig. 2A) in block 8. Trees were planted on double-row beds separated by furrows. Rows were 7.9 m apart with 48 trees spaced 3.9 m apart within rows. To test for lure longevity, we deployed 2 sets of 6 traps baited with pheromone lures (ISCALure-Citrella). The first set was deployed on 24 May 2012. The second set was baited with fresh lures and deployed 8 weeks later on 17 Jul. All traps were monitored until 18 Oct. Lures in traps were not changed during the trial. To control for effects associated with trap location within the block, we randomly rotated traps on each sampling date among 30 positions spread across the block. We assigned 3 positions to each of 10 rows at points that were 37 m, 92 m, and 147 m along the length of each row (183 m). Rows containing traps were

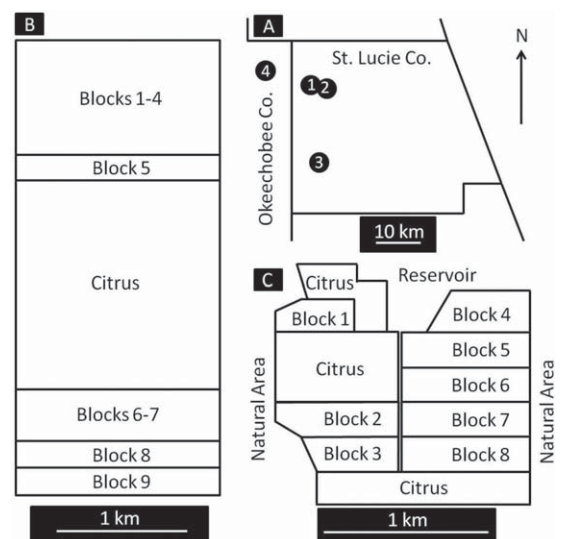


Fig. 2. (A) location of Sites 1-4 in St. Lucie and Okeechobee counties in Florida, (B) relative location of citrus blocks at Site 1, and (C) relative location of citrus blocks at Site 3.

separated by approximately 143 m. We replaced trap liners and rotated traps approximately every 3 days from May through Jun and every 10 days from Jul through Oct. Abundance of *P. insignis* caught per week was averaged within month for each treatment. We evaluated differences in attraction to newly deployed versus aged lures by 2-sample, 2-tailed *t*-test. Data were analyzed using Statistix 9 (Analytical Software 2008). Data are presented as mean  $\pm$  1 standard error of the mean. For *t*-tests, we used a folded *F* test to evaluate homogeneity of variance, and if the result was significant ( $\alpha = 0.05$ ), we used a Satterthwaite approximation (Analytical Software 2008).

#### Seasonal Abundance of *P. insignis* as Measured by Capture Using *P. citrella* Pheromone

We monitored the number of male *P. insignis* caught in delta traps and/or bucket traps during 2012 at 4 sites (1-4) in southeastern Florida (Fig. 2A). For each trap, we calculated the number of moths caught per trap per week and averaged these numbers within each month, based on collection date at each site.

At Site 1, we monitored traps in 8 production blocks (48-56 trees per row, 24.6-32.8 ha per block) of mature 'Marsh Seedless' (blocks 1-7) and 'Flame' (block 9) grapefruit trees (Fig. 2B). From 18 Jan to 24 Apr, we monitored 12 delta traps placed in 6 rows at points 46 m and 137 m along the length of each row (183 m) and 3 bucket traps placed 92 m along the length of 3 other rows in block 9 (see winter/spring trial). We continued to monitor 6 delta traps until 20 Jun. From 27 Apr to 8 Aug, we monitored 26 delta traps placed at points one-third (61-71 m) and two-thirds (122-142 m) along the length of 20 rows (blocks 1-4) or half way (92 m) along the length of 6 rows (block 5), and we continued to monitor 14 delta traps from 29 Aug to 26 Dec in 7 rows at points 61 m and 122 m along the length of each row in block 5 (see blend trial in fall). In blocks 6-7, we monitored one bucket trap per block from 8 Feb to 15 May placed near ends of rows.

At Site 2 (N 27° 28' 37" W 80° 36' 23") in northwestern St. Lucie County, we monitored 15 delta traps from 5 Mar to 14 Jun within a 16 ha block of mature 'Marsh Seedless' grapefruit trees. Rows were 400 m long and spaced 7.6 m apart with approximately 88 trees per row and variable tree spacing within rows. We placed 15 traps within 5 rows spaced 10 rows (76 m) apart. We placed 3 traps within each row at points 100 m, 200 m, and 300 m along the length of each row.

At Site 3 (N 27° 19' 13" W 80° 37' 13") in southwestern St. Lucie County (Fig. 2C), we monitored 13 delta traps from 29 Jun to 26 Dec in 3 production blocks (blocks 1-3, 8.9-13.6 ha) of mature 'Flame' grapefruit trees (Fig. 2C). Rows were 190 m long and spaced 7.2 m apart with 44 trees

spaced 4.3 m apart within rows. We placed 1-2 traps per row at points 57 m and 133 m along the length of each row.

At Site 4 (N 27° 30' 36.7" W 80° 44' 21.6") in northeastern Okeechobee County, we monitored 7 delta traps from 16 Mar to 31 May within a 4.4 ha block of 4 year-old 'Ray Ruby' grapefruit trees. Rows were separated by 7.6 m across bed tops and 9.1 m across furrows. Row length was variable, and trees were spaced 2.24 m apart within rows. We placed 7 traps across 7 rows, spaced 6 rows apart (50 m), and placed half way along the length of each row. Lures in traps were not replaced.

#### Trap Catch Disruption of *P. insignis* with Triene in Winter and Spring

At Site 1 (block 9), we tested the effect of triene (SPLAT CLM) on trap catch disruption in winter and spring (Fig. 2B). We partitioned this production block into treatments according to a 2  $\times$  2 factorial design with split plots in 3 replicated statistical blocks to test the main effect of winter application of triene (treated and untreated) and the subplot effect of spring application of triene. Each replicated block contained 2 main plots (15 beds  $\times$  48 trees, 4.17 ha) that were treated in winter (8 Feb) or left untreated. Main plots were further partitioned into 2 subplots (15 beds  $\times$  24 trees, 2.08 ha), north and south, that were treated with triene during spring (24 Apr) or left untreated. Therefore, after the winter application there were 2 treatments, but after the spring application there were 4 treatments: (1) winter and spring triene, (2) winter triene only, (3) spring triene only, and (4) untreated.

A private applicator (International Fly Masters, Fort Pierce, FL) applied SPLAT CLM containing 0.15% triene (1.5 mg triene per gram of SPLAT CLM) using a tractor-mounted machine that propelled dollops into the tree canopy on both sides of the raised bed. Our target application rate was 750 mg triene/ha (500 g SPLAT CLM/ha). The formulation was dispensed as 1 g dollops, which amounted to approximately 10 dollops per 7 trees, a recommended rate and distribution pattern based on previous research (Lapointe & Stelinski 2011; Lapointe et al. 2011). We collected dollops from trees, and these weighed  $0.84 \pm 0.09$  g ( $n = 7$ ). After the application, the residual weight of SPLAT CLM indicated the true application rate was 564 mg triene/ha (376 g SPLAT CLM/ha). Therefore, we used 60 ml syringes to place additional 1 g dollops on every third tree, bringing the total application rate to 750 mg triene/ha (500 g SPLAT CLM/ha). The same private applicator also applied triene at a rate of 825 mg triene/ha (550 g SPLAT CLM/ha) in spring on 24 Apr.

We evaluated moth catch disruption by placing 2 delta traps baited with pheromone lures (ISCALure-Citrella) near the center of each subplot, in the sixth and tenth beds on 6 Jan before the winter application. We averaged trap catch across main plots before the spring application and across subplots thereafter. We evaluated the effect of triene applied in winter by pooling numbers of moths collected in main plots across 9 weeks (8 Feb to 12 Apr) after application. We analyzed data by 2-sample, 1-tailed *t*-test to test the hypothesis that moth catch was lower in treated compared with untreated plots. We evaluated the effect of triene applied in spring by pooling numbers of moths collected in subplots across 9 weeks (24 Apr to 29 Jun) after application. We analyzed data by factorial ANOVA to test the main effect of winter application and the subplot effect of spring application and their interaction.

#### Trap Catch Disruption of *P. insignis* with Triene or a 3:1 Blend (triene:diene) in Small Plots

We conducted an experiment in mature 'Flame' grapefruit trees at Site 3 (blocks 1-3) to test the effect of triene versus a 3:1 blend of triene:diene on trap catch disruption of *P. insignis*. We partitioned the grove into 0.14 ha plots (5 rows × 9 trees). Each plot was surrounded by a 36 m buffer of trees. We placed a delta trap on the center tree within each plot. We randomly assigned the following treatments to replicated plots: (1) rubber dispensers containing a blend of 2.53 mg triene and 0.84 mg of diene with a distribution of 332 dispensers/ha (840 mg triene +280 mg diene/ha), (2) rubber dispensers containing 2.52 mg triene with a distribution of 332 dispensers/ha (837 mg triene/ha), and (3) untreated control. ISCA Technologies supplied rubber dispensers loaded with the above pheromone contents. This loading dosage has proven effective for mating disruption of *P. citrella* (Stelinski et al. 2008). To treat each plot, rubber dispensers were tied to trees within the exterior canopy 2 m above the ground using 15 cm long pieces of 20 gauge galvanized steel wire (National Manufacturing Company, Cobourg, Ontario, Canada) that were punched through the rubber. We distributed 47 rubber dispensers per plot, which equaled one per tree plus an extra dispenser in the second and fourth rows of each plot. We replicated each rubber dispenser treatment 4 times and the untreated control treatment 13 times. Treatments were applied on 10 Jul.

We evaluated disruption of male *P. insignis* by pooling numbers of moths collected in plots across 13 weeks (13 Jul to 12 Oct). We analyzed data by 1-way ANOVA and orthogonal contrasts to compare: (1) blend and triene treatments combined versus untreated and (2) blend versus triene. To control for family-wide error of orthogonal contrasts, we used a Bonferroni correction, which

established significance at  $\alpha = 0.025$  for each contrast. Data were  $\log(x + 1)$  transformed prior to analysis to improve homogeneity of variance.

#### Trap Catch Disruption of *P. insignis* with a 3:1 Blend (triene:diene) in a Large Plot

We conducted a companion experiment in mature 'Flame' grapefruit trees at Site 3 to test the effect of *P. citrella* pheromone blend (3 triene:1 diene) on trap catch disruption in a 66 ha plot (Fig. 2C, blocks 4-8) compared with trap catch in neighboring untreated 0.14 ha plots (blocks 1-3). The blend was formulated as 1.69 and 0.56 mg of triene and diene (mg/g) of SPLAT, respectively. A private applicator used a prototype machine (Chemical Containers, Lake Wales, FL) mounted on a Kubota RTV1100 that propelled dollops on air streams into the tree canopy on both sides of the raised bed. SPLAT CLM was applied on 10-12 Jul. Dollops collected from trees weighed  $0.84 \pm 0.09$  g ( $n = 8$ ), similar to the weight of dollops applied at Site 1. This application incorporated intentional treatment gaps by skipping 1 bed for every 7 treated, which was shown to reduce the amount of pheromone product needed for mating disruption of *P. citrella* without compromising efficacy (Lapointe and Stelinski 2011). Only the western 10 ha in block 8 were treated. Therefore, the total area treated including intentional gaps was 66 ha, and the application rate across this area was 764 mg triene + 253 mg diene (452 g SPLAT CLM/ha). We monitored trap catch using delta traps baited with pheromone lures targeting *P. citrella* as described above. We placed 15 traps within the treated blocks. Three traps were spread across the treated area within each block (~174 m apart), positioned midway (~96 m) along the length of each row. Traps were monitored from 29 Jun to 15 Aug 2012.

We evaluated trap catch disruption by comparing trap catch in the large (66 ha) plot with the neighboring 13 untreated plots that were part of the small plot trial. Each of the 15 traps in the large plot represented an experimental unit. We compared pretreatment moth abundance using a 2-sample, 2-tailed *t*-test. We compared post-application abundance by pooling numbers of moths collected across 5 weeks (13 Jul to 15 Aug) after treatment using a 2-sample, 1-tailed *t*-test to test the hypothesis that fewer moths were collected in the pheromone-treated plot compared with untreated plots.

#### Trap Catch Disruption of *P. insignis* with a 3:1 Blend (triene:diene) in Fall

We conducted a replicated study at Site 1 (block 5) during the fall to test the effect of a 3:1 blend of triene:diene on trap catch disruption of *P. insignis*. We partitioned the grove into

14 total plots (0.87 ha/plot, 6 rows × 48 trees). Each plot was surrounded by a 0.87 ha buffer of untreated trees. We placed 2 traps in each plot at points 61 and 122 m along the length of the row (183 m), and we monitored these from 29 Aug to 26 Dec. We randomly assigned 2 treatments: (1) rubber dispensers containing a blend of 2.53 mg triene and 0.84 mg diene with a distribution of 331 dispensers/ha (837 mg triene + 278 mg diene/ha), and (2) untreated control. Each treatment was replicated 7 times. Rubber dispensers (ISCA Technologies) were tied to trees on 7 Sep, as described above. Numbers of moths were averaged within plots. We compared pre-treatment moth abundance by 2-sample, 2-tailed *t*-test. After application, we pooled the number of moths collected in traps across 16 weeks (7 Sep-26 Dec) during which time 10 evaluations were made. Across these evaluations, we tabulated how many times we caught at least one *P. insignis* in treated versus untreated plots and analyzed these frequencies by Fisher's Exact Test (SAS Institute 2008).

RESULTS

Lure Attraction and Longevity

No *P. insignis* male moths were captured in unbaited traps throughout the trial (147 days, Fig. 3). In the first set of pheromone-baited traps deployed on 24 May (*n* = 6 per treatment), we caught 42 moths during the length of the trial. We caught 1.4 ± 0.9 male *P. insignis* moths per trap per week (moths/trap/week ± 1 SEM) in late May. Male moths continued to respond to the pheromone in Jun (0.8 ± 0.3), Jul (0.6 ± 0.2),

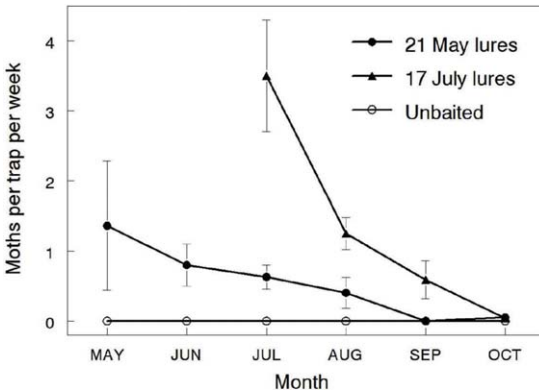


Fig. 3. Mean (± 1 SEM) number of male *Phyllocnistis insignis* caught in a citrus grove in unbaited delta traps or those baited with a 3:1 blend of (Z,Z,E)-7,11,13-hexadecatrienal (triene) and (Z,Z)-7,11-hexadecadienal (diene) on 21 May or 17 Jul until 18 Oct. Abundance of *P. insignis* was averaged within month for each treatment (*n* = 6).

and Aug (0.4 ± 0.2). These traps caught no moths in Sep, but a few moths (0.05 ± 0.05) were caught in Oct (Fig. 3). In the second set of pheromone-baited traps deployed on 17 Jul (*n* = 6 per treatment), we caught 62 moths during the length of the trial. We caught 3.5 ± 0.8 moths/trap/week in Jul. Male moths continued to respond to the pheromone in Aug (1.2 ± 0.2) and Sep (0.6 ± 0.3) before falling to just 0.04 ± 0.04 moths/trap/week in Oct (*n* = 5). From 17-26 Jul, traps with newly deployed *P. citrella* lures (3.5 ± 0.8 moths/trap/week) caught over 4 times the number of *P. insignis* moths caught in traps baited with 8 week-old lures (0.8 ± 0.3 moths/trap/week, *t*<sub>6,3</sub> = 3.2, *P* = 0.02).

Seasonal Abundance of *P. insignis* as Measured by Capture Using *P. citrella* Pheromone

Bucket and delta traps were equally effective at trapping *P. insignis* (Fig. 4A). The abundance of moths caught in traps peaked in May at 2.1 ± 0.1 and 1.4 ± 0.1 moths/trap/week at Site 1 in bucket and delta traps, respectively. The peak catch in May was 1.1 ± 0.1 at Site 2 and reached 4.5 ± 0.5 moths/trap/week at Site 4 (Fig. 4B). There were smaller peaks in Feb and Aug. The abundance of moths in traps remained below 0.2 moths/trap/week from Sep through Dec (Site 3, Fig. 4B).

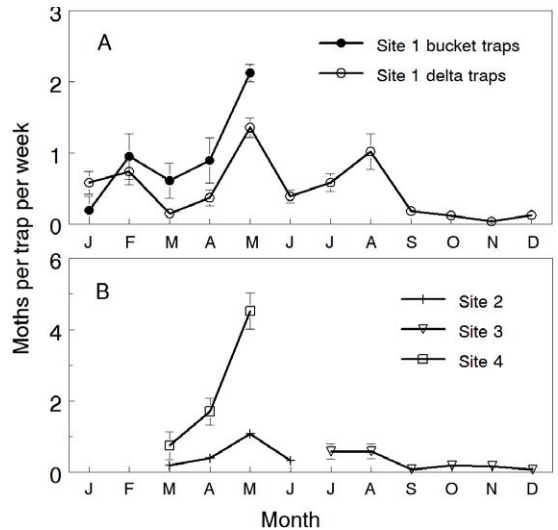


Fig. 4. Mean (± 1 SEM) number of *Phyllocnistis insignis* males caught in citrus groves (A) at Site 1 in bucket or delta traps, and (B) at Sites 2-4 in delta traps. The number of moths per trap per week was averaged within months for each site or trap type [Site 1 bucket traps, *n* = 3, 5, 5, 5, 2 (Jan-May, respectively); Site 1 delta traps, *n* = 12, 12, 12, 12, 32, 32, 26, 26, 14, 14, 14, 14 (Jan-Dec, respectively); Site 2, *n* = 15; Site 3, *n* = 14; Site 4, *n* = 13].

#### Trap Catch Disruption of *P. insignis* with Triene in Winter and Spring

We caught  $1.2 \pm 0.3$  (untreated plots) and  $1.1 \pm 0.3$  (treated plots) male *P. insignis* moths/trap/week from 18 Jan to 8 Feb, prior to treatment with triene (SPLAT CLM). Two weeks after application of triene in winter, we caught no moths in treated plots and  $0.25 \pm 0.07$  moths/trap/week in untreated plots. Triene applied during winter did not influence the number of moths caught in traps during a 9 week period after application ( $t_4 = -0.21$ ,  $P = 0.42$ ). Triene applied in winter also did not influence catch of *P. insignis* after spring application of triene (ANOVA,  $F_{1,2} = 3.31$ ,  $P = 0.21$ ) or interact with the spring application (ANOVA,  $F_{1,4} = 3.25$ ,  $P = 0.15$ ). However, spring application of triene reduced catch of *P. insignis* compared to untreated plots (ANOVA,  $F_{1,4} = 34.8$ ,  $P < 0.01$ , Fig. 5). We collected half as many moths in treated plots ( $0.4 \pm 0.1$  moths/trap/week) as in untreated plots ( $1.0 \pm 0.2$  moths/trap/week) during a 9 week time period after application in spring.

#### Trap Catch Disruption of *P. insignis* with Triene or a 3:1 Blend (triene:diene) in Small Plots

We caught one-tenth as many *P. insignis* males in blend-treated ( $0.04 \pm 0.02$  moths/trap/week) or triene-treated ( $0.04 \pm 0.04$  moths/trap/week) plots compared with untreated plots ( $0.4 \pm 0.1$  moths/trap/week) during a 13 week period after treatment (ANOVA,  $F_{2,18} = 4.75$ ,  $P = 0.022$ , Table 1). Fewer moths were caught in pheromone treated plots compared with the untreated control (orthogonal contrast,  $t = -3.1$ ,  $P = 0.0065$ ). The

number of moths caught in triene-treated versus blend-treated plots did not differ (orthogonal contrast,  $t = 0.12$ ,  $P = 0.91$ , Table 1).

#### Trap Catch Disruption of *P. insignis* with a 3:1 Blend (triene:diene) in a Large Plot

The abundance of *P. insignis* moths caught before applying the pheromone blend did not differ between the 66 ha plot and neighboring untreated small plots ( $t_{21.2} = 1.4$ ,  $P = 0.19$ , Table 1). However, after applying the pheromone blend, we caught one-quarter the number of moths in treated ( $0.2 \pm 0.1$  moths/trap/week) versus untreated ( $0.8 \pm 0.3$  moths/trap/week) plots during a 5 week period ( $t_{13.7} = -2.14$ ,  $P = 0.025$ , Table 1).

#### Trap Catch Disruption of *P. insignis* with a 3:1 Blend (triene:diene) in Fall

The abundance of moths caught before applying the pheromone blend did not differ between treated ( $0.22 \pm 0.12$  moths/trap/week) and untreated ( $0.33 \pm 0.13$  moths/trap/week) plots ( $t_{12} = -0.63$ ,  $P = 0.54$ ,  $n = 7$  per treatment). After applying the pheromone blend, we caught no moths in treated plots and caught  $0.10 \pm 0.03$  moths/trap/week (22 moths total, range 0-3 moths/trap/evaluation) in untreated plots over a 16-week period, during which there were 10 moth evaluations. Across these evaluations, the incidence of catch was lower in treated than in untreated plots (Fisher's Exact Test,  $P = 0.01$ ). We did not catch *P. insignis* in treated plots compared with 6 incidents of catch in untreated plots.

## DISCUSSION

Male *P. insignis* moths in Florida were attracted to delta traps and bucket traps baited with a 3:1 blend of (*Z,Z,E*)-7,11,13-hexadecatrienal and (*Z,Z*)-7,11-hexadecadienal, the 2 major components of pheromone produced by the invasive *P. citrella* (Leal et al. 2006). Lures remained attractive 20 weeks after deployment, but attraction appeared to diminish over time as pheromone release diminished, since traps with newly deployed lures caught more moths than lures that were 8 weeks old. We trapped *P. insignis* moths throughout the year in Florida. The population peaked in May with minor peaks in Feb and Aug before declining to low numbers during fall. *Phyllocnistis insignis* may oviposit and develop year round on American burnweed, an annual herb that flowers year round in central Florida (Minno 1992; Weekley et al. 2006). We found this plant growing along ditches between rows of citrus at Site 3 on 12 Feb 2013, but no mines were found on leaves. We were unable to find either American burnweed or butterweed elsewhere in St. Lucie and Okeechobee

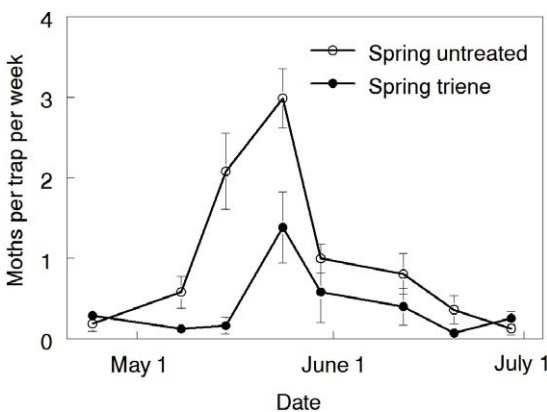


Fig. 5. Mean ( $\pm 1$  SEM) number of *Phyllocnistis insignis* moths per trap per week collected in delta traps in untreated plots or those treated with 825 mg triene/ha (550 g SPLAT CLM/ha) in spring (24 Apr). Fewer moths were caught in plots treated with triene during a 9 week period (24 Apr to 29 Jun) after application in spring (ANOVA,  $F_{1,4} = 34.8$ ,  $P < 0.01$ ) than in untreated plots.



TABLE 1. MEAN ( $\pm$  1 SEM) NUMBER OF *PHYLLOCNISTIS INSIGNIS* MOTHS CAUGHT IN SMALL PLOTS TREATED WITH TRIENE (837 MG/HA), BLEND (840 MG TRIENE + 280 MG DIENE/HA) PHEROMONE, OR LEFT UNTREATED AND MOTH CATCH IN A LARGE NEIGHBORING PLOT TREATED WITH BLEND (764 MG TRIENE + 253 MG DIENE/HA) PHEROMONE.

| Week        | Delta trap collection | Moths per trap per week  |                 |                 |                         |               |                             | Statistical comparison <sup>c</sup> |
|-------------|-----------------------|--------------------------|-----------------|-----------------|-------------------------|---------------|-----------------------------|-------------------------------------|
|             |                       | Small plots <sup>a</sup> |                 |                 | Large plot <sup>b</sup> |               |                             |                                     |
|             |                       | Untreated                | Triene          | Blend           | Blend                   | Blend         | Blend                       |                                     |
| Pre         | 6/29 - 7/12           | 1.1 $\pm$ 0.4            | —               | —               | —                       | 2.1 $\pm$ 0.6 | $t_{21,2} = 1.4, P = 0.19$  |                                     |
| 1           | 7/13 - 7/19           | 0.5 $\pm$ 0.2            | 0               | 0.3 $\pm$ 0.3   | —                       | 0             | —                           |                                     |
| 2           | 7/19 - 7/26           | 0.8 $\pm$ 0.5            | 0               | 0               | 0                       | 0.1 $\pm$ 0.1 | —                           |                                     |
| 3           | 7/26 - 8/03           | 1.3 $\pm$ 0.6            | 0               | 0.2 $\pm$ 0.2   | —                       | 0.4 $\pm$ 0.4 | —                           |                                     |
| 4           | 8/03 - 8/09           | 0.3 $\pm$ 0.2            | 0               | 0               | —                       | 0.2 $\pm$ 0.1 | —                           |                                     |
| 5           | 8/09 - 8/15           | 0.7 $\pm$ 0.3            | 0               | 0               | —                       | 0.2 $\pm$ 0.1 | —                           |                                     |
| 7           | 8/15 - 8/31           | 0.1 $\pm$ 0.1            | 0               | 0               | —                       | —             | —                           |                                     |
| 9           | 8/31 - 9/12           | 0.2 $\pm$ 0.1            | 0               | 0               | —                       | —             | —                           |                                     |
| 10          | 9/12 - 9/19           | 0                        | 0               | 0               | —                       | —             | —                           |                                     |
| 11          | 9/19 - 9/26           | 0.1 $\pm$ 0.1            | 0.3 $\pm$ 0.3   | 0               | —                       | —             | —                           |                                     |
| 13          | 9/26 - 10/12          | 0.6 $\pm$ 0.2            | 0.1 $\pm$ 0.1   | 0               | —                       | —             | —                           |                                     |
| Pooled data |                       |                          |                 |                 |                         |               |                             |                                     |
|             | 7/13 - 8/15           | 0.8 $\pm$ 0.3            | —               | —               | —                       | 0.2 $\pm$ 0.1 | $t_{13,7} = 2.1, P = 0.025$ |                                     |
|             | 7/13 - 10/12          | 0.4 $\pm$ 0.1            | 0.04 $\pm$ 0.04 | 0.04 $\pm$ 0.02 | —                       | —             | $F_{2,18} = 4.8, P = 0.022$ |                                     |

<sup>a</sup>Randomized 0.14 ha plots treated 10 Jul with triene ( $n = 4$ ) or blend ( $n = 4$ ) from rubber dispensers or untreated [ $n = 9$  (6/29-7/12),  $n = 13$  (7/13-10/12)]; each plot contained 1 trap.

<sup>b</sup>66 ha plot treated 10-12 Jul with blend ( $n = 15$ ).

<sup>c</sup>Treatments compared within rows: pre-application data (6/29 - 7/12) analyzed by 2-sample, 2-tailed t-test; post-application pooled data (7/13 - 8/15) analyzed by 2-sample, 1-tailed t-test. Small plot data analyzed by 1-way ANOVA and orthogonal contrasts to compare: (1) untreated versus pheromone-treated plots ( $t = 3.1, P = 0.0065$ ) and (2) triene versus blend ( $t = 0.12, P = 0.91$ ).

counties where these experiments were conducted and along edges of citrus groves.

Application of *P. citrella* pheromone components in citrus groves disrupted catch of *P. insignis* in pheromone-baited traps in 3 replicated trials. In the first trial (Site 1, block 9), application of triene in spring disrupted catch of *P. insignis* during a period of increasing moth abundance in May. However, application of the same triene formulation at a 10% lower rate did not disrupt trap catch during winter, which may be attributed to the low population in control plots at the site in late Feb and Mar. In the second trial (Site 3), application of triene or a 3:1 blend (triene:diene) released from rubber dispensers disrupted catch of *P. insignis* in randomized 0.14 ha plots during summer and fall. Likewise, application of a 3:1 blend (triene:diene) released from SPLAT CLM applied to a 66 ha plot as part of a companion trial disrupted catch of *P. insignis* compared with neighboring untreated plots. Disruption was particularly evident when the population was abundant in control plots. In the third trial (Site 1, block 5), application of a 3:1 blend (triene:diene) released from rubber dispensers in 0.87 ha plots reduced the incidence of *P. insignis* to zero during a 16 week period in late summer and fall when moth abundance was particularly low.

Species within the genus *Phyllocnistis* may share pheromone components, as do other closely related Lepidoptera (Ando et al. 2004; Inomata et al. 2005; Mozüraitis et al. 2008). Male *P. insignis* may be attracted to one or both pheromone components of *P. citrella*. The diene alone attracts the congener *P. wampella* Liu & Zeng in China (Du et al. 1989). In pheromone-baited bucket traps, we have distinguished several phenotypes that may be separate species. These may include *P. viti-foliella* Clemens or *P. vitegenella* Clemens from wild grape (*Vitis rotundifolia* Michx.) plants that are abundant near citrus groves in south Florida (USDA-NRCS 2012).

The impact of mating disruption on non-target species has not received sufficient investigation (Martinez & Mgocheki 2012). The effect could be beneficial if non-target species are pests, or detrimental if they serve as reservoirs or food sources for biological control agents (Rizzo et al. 2006). For example, pheromone components released to control *P. citrella* could reduce mating success of other *Phyllocnistis* species, and this could influence populations of *P. citrella* indirectly if the non-target species serve as alternative hosts for native or introduced parasitoids of *P. citrella*. Pheromone mixtures that target multiple pests are finding utility because of the cost savings for growers who face a complex of pests that directly affect fruit quality and/or production (Deland et al. 1994; Stelinski et al. 2009; Suckling et al. 2012). This approach broadens the potential for mating disruption on non-target species.

Our results confirmed trap catch disruption of the non-target *P. insignis* with application of mating disruption against *P. citrella* in citrus groves. This phenomenon leads to questions of species interaction in citrus groves and suggests that a more complete understanding of species within the *Phyllocnistis* genus and other members of the Gracillariidae and their natural enemies could provide a broader context for pest management decisions. The impact of mating disruption on non-target species, be it positive or negative, is an area of research that deserves further attention.

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