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Disruption of the leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in citrus: effect of blend and placement height, longevity of disruption and emission profile of a new dispenser

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Abstract

Recent efforts to disrupt mating of the leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), a global pest of citrus, have focused on the use of SPLAT™ (ISCA Technologies), a flowable wax emulsion intended to serve as a slow-release matrix for pheromones. Early success with this approach was overshadowed by the expense and difficulty of application, and variation in wax component chemistry that contributed to reduced longevity of pheromone emission in the field. Solid elastomer dispensers (DCEPT CLM™, ISCA Technologies, Inc.) loaded with a 3:1 blend of (*Z,Z,E*)-7,11,13-hexadecatrienal and (*Z,Z*)-7,11-hexadecadienal, the major components of the *P. citrella* sex pheromone, provided disruption of trap catch in commercial citrus orchards for periods exceeding 30 wk. The triene component alone worked as well as or better than the 3:1 blend. The height of dispensers placed by hand in the tree canopy had a significant effect on trap shutdown. Dispensers placed low (0.6 m) in the canopy resulted in a reduction of trap shutdown in the upper third (>3 m) of the canopy suggesting that the net movement of pheromone molecules was downward during the period of active moth flight. Although moth flight appeared equivalent among the heights tested, placement of dispensers higher in the canopy appears more effective given this downward movement of pheromone plumes. These studies suggest that season-long trap catch disruption can be attained in citrus with a single application of a hand-applied dispenser.

Key Words*:* sex pheromone; (*Z,Z,E*)-7,11,13-hexadecatrienal; citrus leafminer; SPLAT; DCEPT CLM

Resumen

Trabajos recientes por impedir el apareamiento del minador de la hoja, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), una plaga mundial de cítricos, se han centrado en el uso de SPLAT ™ (ISCA Technologies), una emulsión de cera fluida diseñado a servir como una matriz de liberación lenta para feromonas. El éxito inicial con este enfoque se vio ensombrecida por el costo y la dificultad de aplicación, y variación en la composición química que contribuyó a la reducción de la longevidad de la emisión de feromonas en el campo. Dispensadores de goma sólida (DCEPT CLM ™, ISCA Technologies, Inc.) cargados con una mezcla 3:1 de (*Z, Z, E*)-7,11,13 hexadecatrienal:(*Z, Z*)-7,11-hexadecadienal, los principales componentes de la feromona sexual de *P. citrella*, proporcionaron excelente reducción en la captura de machos en trampas en huertos comerciales de cítricos por períodos superiores a 30 semanas. El componente trieno funcionaba iqual o mejor que la mezcla 3:1. La altura de los dispensadores colocados a mano en los árboles tuvo un efecto significativo sobre la captura en trampas. Dispensadores colocados bajo (0,6 m) resultaron en una reducción de disrupción de captura en el tercio superior (>3 m) de los arboles lo que sugiere que el movimiento neto de moléculas de feromona fue hacia abajo durante el período de vuelo de la polilla. Aunque el vuelo de la polilla apareció equivalente entre las alturas probadas, la colocación a 3 m de dispensadores en la copa parece más eficaz dado a este movimiento de los penachos de feromonas. Estos estudios sugieren que la interrupción durante toda la temporada se puede lograr en los cítricos con una sola aplicación de un dispensador aplicado a mano y proporcionamos recomendaciones para su utilización.

Palabras Clave: feromona sexual; (*Z,Z,E*)-7,11,13-hexadecatrienal; minador de las hojas de los cítricos; SPLAT; DCEPT CLM

Recent efforts (Lapointe et al. 2009, 2014; Stelinski et al. 2010) to provide economical and effective control of the leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), a pest of citrus crops throughout the world (Heppner 1993), have focused on the use of SPLAT™ (ISCA Technologies, Riverside, California, USA) as a slow release matrix for sustained pheromone delivery for the purpose of mating disruption. Damage caused by this leafminer reduces leaf photosynthetic capacity and produces wounds that serve as sites of infection for citrus

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bacterial canker, *Xanthomonas axonopodis* pv. citri (Xanthomonadales: Xanthomonadaceae) resulting in greater incidence and severity of the disease (Gottwald et al. 2007; Hall et al. 2010). Successful disruption of *P. citrella* in commercial citrus (Stelinski et al. 2008) was demonstrated with SPLAT CLM™, an emulsified wax formulation containing an off-ratio blend of *P. citrella* sex pheromone over several weeks (Lapointe & Stelinski 2011). A tractor-mounted applicator was developed to deliver dollops of SPLAT into citrus canopies (Lapointe & Stelinski 2011), and studies have shown that coverage patterns can be varied to reduce cost while maintaining efficacy of trap disruption (Lapointe et al. 2014).

The cost of mating disruption of *P. citrella* is a function of several factors including the cost of synthesis of the pheromone, the delivery device, and application technology. Synthesis of the primary component of the *P. citrella* sex pheromone, (*Z,Z,E*)-7,11,13-hexadecatrienal, involves a pyrophoric reagent (Leal et al. 2006; Moreira et al. 2006) that constrains the scale of synthesis and contributes significantly to the price of any pheromone release device. In the case of the tractor-mounted applicator developed for SPLAT (Lapointe & Stelinski 2011), application costs include the cost of operation and transport of the equipment and the operator's time as well as the initial cost of fabrication of the specialized machinery. Here we report studies that document the efficacy of hand-applied solid dispensers (DCEPT CLM™, ISCA Technologies, Riverside, California, USA) for disruption of *P. citrella* attraction to pheromone-baited traps in commercial citrus operations in Florida. Disruption of trap catch in traps baited with the attractive sex pheromone of a moth is a proxy for mating disruption that is more difficult to measure in the field. The objective of mating disruption in the case of *P. citrella* is to reduce mining and damage. The objective of this study was to document the ability of a release device to deliver pheromone under actual field conditions in Florida, USA. An effective, long-lived release device is a necessary step in the development of a mating disruption technique for *P. citrella*. In addition, we compared a "natural" 3:1 blend of (*Z,Z,E*)-7,11,13-hexadecatrienal:(*Z,Z*)-7,11-hexadecadienal with a triene-only formulation, studied the effect of device and trap placement in grapefruit tree canopies on trap catch, and generated a pheromone release profile for the new device.

Materials and Methods

FIELD TRIALS

A prototype solid elastomer dispenser (DCEPT CLM™, ISCA Technologies Inc., Riverside, California, USA) was applied in 3 commercial citrus groves during 2012: VPI-5, Golden River Fruit Company (27°19'N, 80°37'W) in southwestern St. Lucie County, Florida; Emerald Grove (27°28'N, 80°38'W), The Packers of Indian River, in northwestern St. Lucie County, Florida; and TRB Groves (27°01'N, 81°46'W) in northern Charlotte County, Florida. A trial was established at the Emerald Grove location using dispensers loaded with a 3:1 blend (1.0:0.33 mg) of (*Z,Z,E*)-7,11,13-hexadecatrienal:(*Z,Z*)-7,11-hexadecadienal. The dispenser measured 1.2 cm in diameter and was fitted in a white plastic hanger for placement in trees (Fig. 1). Plots consisted of 6 rows of mature grapefruit with 48 trees per row. Tree spacing was 8 m between rows and 3.8 m between trees within rows in a double bed planting

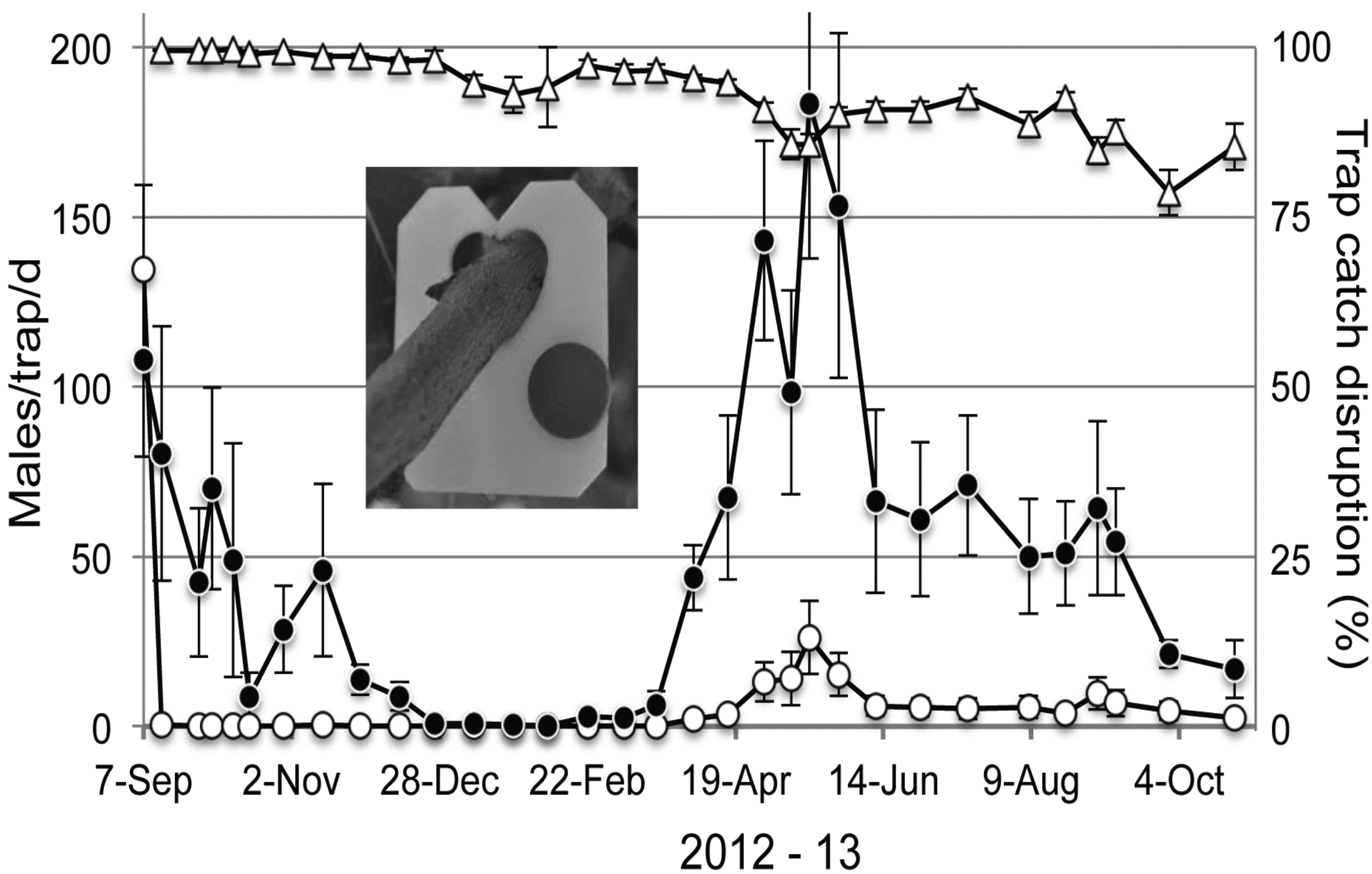

Fig. 1. Mean ± SEM number of male *Phyllocnistis citrella* captured in pheromone-baited traps in untreated control plots (filled circles, *n* = 9) and in pheromone-treated plots (open circles, *n* = 14) of grapefruit at Emerald grove, St. Lucie County, Florida, USA. Triangles are mean ± SEM (*n* = 14) percentage trap catch disruption (right y axis). Insert: DCEPT CLM dispenser. Rubber disk is 1.2 cm in diameter; white plastic hanger is 3.5 × 4.3 cm.

configuration. Plot size was approximately 0.9 ha with buffer zones of identical size between plots. Treatments consisted of treated and untreated plots in a completely randomized design with 7 replicates. In the treated plots, one dispenser was hung in each tree at a height of approximately 2 m above the ground. Two traps were placed equidistant from each other and the row ends in the center row of each plot at 2 m above the ground to assess trap shutdown. Devices were deployed in early Sep 2012; adhesive trap liners were monitored weekly through Oct 2013.

A second trial was established at the VPI-5 location with dispensers loaded with 1.33 mg of a 3:1 (*Z,Z,E*)-7,11,13-hexadecatrienal:(*Z,Z*)-7,11-hexadecadienal blend, or 1 mg of the triene component alone so that both dispenser formulations contained the same initial amount of the triene. One dispenser was hung in each tree at a height of approximately 2 m above the ground. Tree spacing was 7.3 m between rows and 4.3 m between trees within rows in a double bed planting configuration. Plots were approximately square and consisted of 5 rows of 9 trees each (36.5 × 38 m, 0.14 ha). Plots were arranged with buffer zones between plots consisting of 36.5 m (5 untreated rows) between plots across rows and 38 m (9 untreated trees) between plots within rows. Treatments (blend, triene only, or untreated control) were assigned in a completely randomized design with 4 replications. A single trap was placed in the center of each plot to assess trap shutdown.

A third field trial was conducted at TRB grove in Charlotte County, Florida, during 2012–2013. Approximately 42.5 ha (1,200 × 354 m) of grapefruit (red grapefruit scion on Swingle rootstock) were treated with rubber pheromone dispensers as described above in late Aug 2012. Tree spacing was 3.7 m between trees within rows and 7.5 m between rows in double-row beds. Traps were deployed in the center of each of 6 rows spaced approximately 96 m apart in the treated grapefruit and in 2 blocks of equivalent size of untreated Hamlin orange trees located to the north and south of the treated grapefruit block for comparison and calculation of percent trap catch disruption. Trap liners were collected and counted approximately weekly through Dec 2013.

Pherocon VI traps (Trécé, Adair, Oklahoma, USA) baited with a rubber septum loaded with 0.1 mg of (*Z,Z,E*)-7,11,13-hexadecatrienal and 0.033 mg of (*Z,Z*)-7,11-hexadecadienal (IT203, ISCAlure-Citrella™, ISCA Technologies), were placed in the center rows of treated and untreated plots to assess trap shutdown. Sticky trap liners were replaced and counted approximately weekly as grove conditions and weather allowed at each of the 3 sites.

EFFECT OF DISPENSER AND TRAP HEIGHTS

A 2-factor 3 × 3 factorial design with 4 repetitions was used to examine the effect of vertical placement of both trap and pheromone dispenser on trap catch in a grapefruit grove in northwestern St. Lucie County (Kentucky 4, The Packers of Indian River), Florida. Sixteen plots were arranged in a 4 × 4 grid in a 16 ha grove with double-row beds. Plots consisted of approximately square areas (45 × 45 m = 0.2 ha) of 6 rows of grapefruit with 9 trees per row. Plots received 1 of 3 dispenser treatments and a control (no pheromone dispenser) in a completely randomized design with 45 m of untreated (buffer) area between all plots. Within each plot, 3 Pherocon VI traps were placed in the center bed (rows 3 and 4), one at each of 3 heights. Traps and dispensers were hung at ≤ 0.6, ~ 1.8, or ≥ 3 m in the 3rd, 5th, and 7th tree of the center bed. Trap height was randomly assigned to tree position within the row. Traps were baited with one IT203 lure for the duration of the experiment. Sticky trap liners were changed approximately weekly as grove conditions and weather allowed over a period of 11 wk from 30 Oct 2013 through 14 Jan 2014. Meteorological data were downloaded from the Florida Automated Weather Network (FAWN) for the St. Lucie West location, approximately 13 km in a straight line from the location of the height experiment.

DISSIPATION OF PHEROMONE

One hundred dispensers were placed at 2 m above the ground on branches of mature citrus trees at the United States Department of Agriculture–Agricultural Research Service (USDA–ARS) experiment farm near Fort Pierce, Florida, in Apr 2014. Ten rubber dispensers loaded by the manufacturer with 2.7 mg of (*Z,Z,E*)-7,11,13-hexadecatrienal were placed in each of 10 trees. Ten dispensers, one from each of the 10 trees, were collected on each sample date, transported to a laboratory and kept at −20 °C until extracted to determine the amount of (*Z,Z,E*)-7,11,13-hexadecatrienal remaining in the dispenser. Dispensers were placed in individual 25 mL glass vials with 10 mL of hexane containing 25 ng/µL hexadecanal as an internal standard. Vials were sealed and kept at 22 °C without agitation for 24 h. Dilutions (1:10) of the initial extracts were prepared by adding 100 µL of extract to 900 µL of extraction solution previously transferred into a 2 mL autosampler vial. Re-extraction with a second portion of the extraction solution yielded no appreciable additional concentration of pheromone, verifying that a single extraction was sufficient to determine the pheromone in the dispensers.

A stock standard was prepared by dissolving 5.1 mg of (*Z,Z,E*)-7,11,13-hexadecatrienal in a 10 mL volumetric flask brought to volume with extraction solution. Calibration standards were prepared from the stock by transferring 500, 200, 100, and 50 µL aliquots to 10 mL volumetric flasks and bringing to volume with extraction solution. Standards and samples were analyzed with a ThermoScientific DSQII single quadrupole mass spectrometer interfaced with a Trace Ultra gas chromatograph equipped with a programmable temperature and volume (PTV) injector operated in the constant temperature (CT) splitless mode. Three control samples were analyzed with each batch of 10 dispensers: a blank rubber dispenser without pheromone, and 2 laboratory-fortified blank dispensers, which were the same rubber dispenser as the blank but with 200 µL of stock solution added in 100 µL aliquots and allowed to evaporate before extraction. The column used was a DB-5MS (30 m × 0.25 mm ID with a 0.25 µ film) (Agilent Technologies, Santa Clara, California, USA). Sample injection was performed by a ThermoScientific TriPlus RSH robotic autosampler. Instrument control, data acquisition, and data processing were all accomplished using Xcalibur software (Thermo Scientific, USA). Chromatographic column run conditions were temperature-programmed starting at 40 °C with a 4 min hold, then 8 °C/min to 200 °C with a 10 min hold. The MS transfer line was maintained at 250 °C throughout the run. Electronic pressure control maintained helium carrier gas column flow at 1 mL/min throughout the run. One µL injections were made with the injector port at 220 °C for 3 min. The mass spectrometer started collecting data 8.5 min into the run and scanned from m/z 40 to m/z 450. The system was tuned prior to each sequence, and all other mass spectral conditions were taken from the tune file. Source temperature was maintained at 220 °C. A full calibration curve including extraction solution as a calibration blank was run at the start of each analytical sequence. Calibration was verified immediately after the curve was run prior to any samples being analyzed, and during the sequence by re-running the third calibration standard after every 7 to 10 samples. The analyte and ISTD responses were measured in all standards and samples using extracted ion current profiles (EICPs), the former at m/z 95 and the latter at m/z 222. The correlation coefficient (R^2) for all calibration curves was > 0.999; analyte concentrations in the Continuing Calibration Verification (CCV) standards were within 15% of known concentrations; continuing calibration blanks (CCBs) showed no detectable analyte; and analyte recovery in laboratory-fortified blanks was between 90 and 110%.

For the purpose of comparison, we plotted the emission data for DCEPT CLM dispensers along with equivalent data previously published for SPLAT CLM™ from a study conducted under very similar conditions in central Florida (Stelinski et al. 2010).

Statistical Analyses

Counts of male *P. citrella* caught on liners were standardized by dividing trap catch by the number of days the liners were deployed. Trap catch disruption was calculated as the proportion of trap catch in pheromone-treated plots versus control plots [% trap catch disruption = 100 × (1 − trap catch in treated plot/trap catch in control plots)]. Percent trap catch disruption was transformed by the angular (arcsine) transformation as necessary to stabilize variance prior to analysis. Untransformed means are reported in the text and figures. Treatment effects and interactions were compared by analysis of variance (ANOVA) and, when appropriate, means were compared by Tukey's HSD test ($\alpha = 0.05$).

Results

FIELD TRIALS

Trap catch disruption was high (> 95%) and persisted for several months after deployment of dispensers at both locations. Application of dispensers containing the 3:1 blend at the Emerald Grove location occurred on 7 Sep 2012 and resulted in trap catch disruption of > 98% for 16 wk and >93% trap disruption for 32 wk after deployment (Fig. 1). Disruption became more variable during the winter months when trap catch in untreated plots was < 1 male per trap per day (28 Dec through 22 Feb). Disruption persisted through the spring and summer of 2013 at > 85% for 52 wk and was still > 75% through the end of the trial at 59 wk after deployment of the release devices (Fig. 1). At 1 yr after deployment, trap catch disruption (trap shutdown) was still significant ($\alpha = 0.05$, 2-tailed *t*-test of trap catch in treated vs. untreated plots).

At both field locations, *P. citrella* populations were high; trap catch in untreated control plots often exceeded 50 males per trap per day during population peaks (Figs. 1 and 2). The comparison of blend and triene dispensers was deployed in Jul 2012 at VPI-5 grove and included dispensers loaded with the "natural" 3:1 blend of triene:diene or loaded with the triene component only with the same amount of triene as in the blend. At VPI-5, both devices provided ≥ 95% trap disruption for 22 wk after deployment (Fig. 2). Disruption became more variable during the winter months when trap catch in untreated plots declined to very low levels, particularly from 26 Dec through 12 Feb when trap catch in untreated plots was < 1 male per trap per day (Fig. 2). There was no significant difference in percentage trap catch disruption between pheromone formulations ($F = 0.0098$, Pr > $F = 0.92$) and no significant interaction between formulation and date for that variable ($F = 0.0024$, Pr > $F = 0.96$). Therefore, the mean trap catch and trap catch disruption for the combined treatments (blend and triene) are presented in Fig. 2. Disruption declined in the spring of 2013, presumably due to depletion of pheromone in the dispensers but still averaged 81 ± 4.4% on the date of greatest spring flight on 19 Apr at 40 wk after deployment of the dispensers (Fig. 2).

Results at the TRB site in Charlotte County were similar to those obtained at the other 2 sites. Trap catch in the grapefruit block treated with pheromone dispensers was consistently low during the fall of 2012 and throughout 2013 (Fig. 3) compared with the trap catch in the untreated blocks of Hamlin orange to the north and south. Trap catch disruption exceeded 95% for the entire period.

EFFECT OF DISPENSER AND TRAP HEIGHTS

Trap catch of male *P. citrella* in untreated plots varied over the 7 sampling dates (weeks) of the experiment from 3.4 ± 3.1 to 30.7 ± 3.1 males per trap per day ($F = 12.84$; df = 6, 83; Pr > $F < 0.0001$). There was no significant effect of trap height on trap catch in the untreated plots ($F = 0.14$; df = 2, 12; Pr > $F = 0.87$) and no interaction between trap height and date ($F = 0.41$; df = 22, 83; Pr > $F = 0.87$). Mean (± SEM, n = 28) trap catch was 17.2 ± 3.3, 15.7 ± 2.7, and 14.3 ± 1.8 moths per trap per day at 0.6, 2.0, and > 3.0 m, respectively.

There was no significant effect of sample date on percentage trap catch disruption (with or without the angular transformation) over the 7 weekly sampling dates (untransformed data: $F = 1.967$; df = 6, 245; Pr > $F = 0.071$); disruption ranged from 86 to 94 ± 2.1% (pooled SEM, n = 251). There was a significant interaction between dispenser height and trap height ($F = 7.1741$; df = 2, 243; Pr > $F < 0.0001$). Therefore,

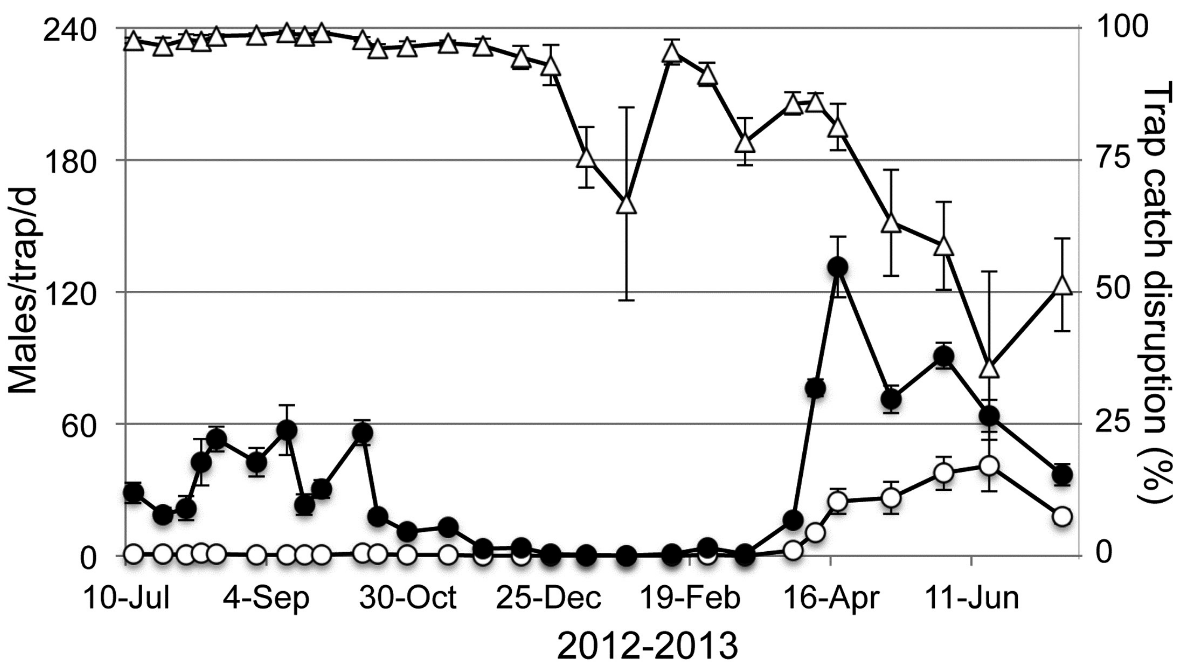

Fig. 2. Mean ± SEM (n = 14) number of male *Phyllocnistis citrella* captured in pheromone-baited traps in untreated control plots (filled circles) and in pheromone-treated plots (open circles) of grapefruit at VPI-5 grove, St. Lucie County, Florida, USA (n = 8). Triangles represent mean ± SEM (n = 8) percentage trap catch disruption (right y axis).

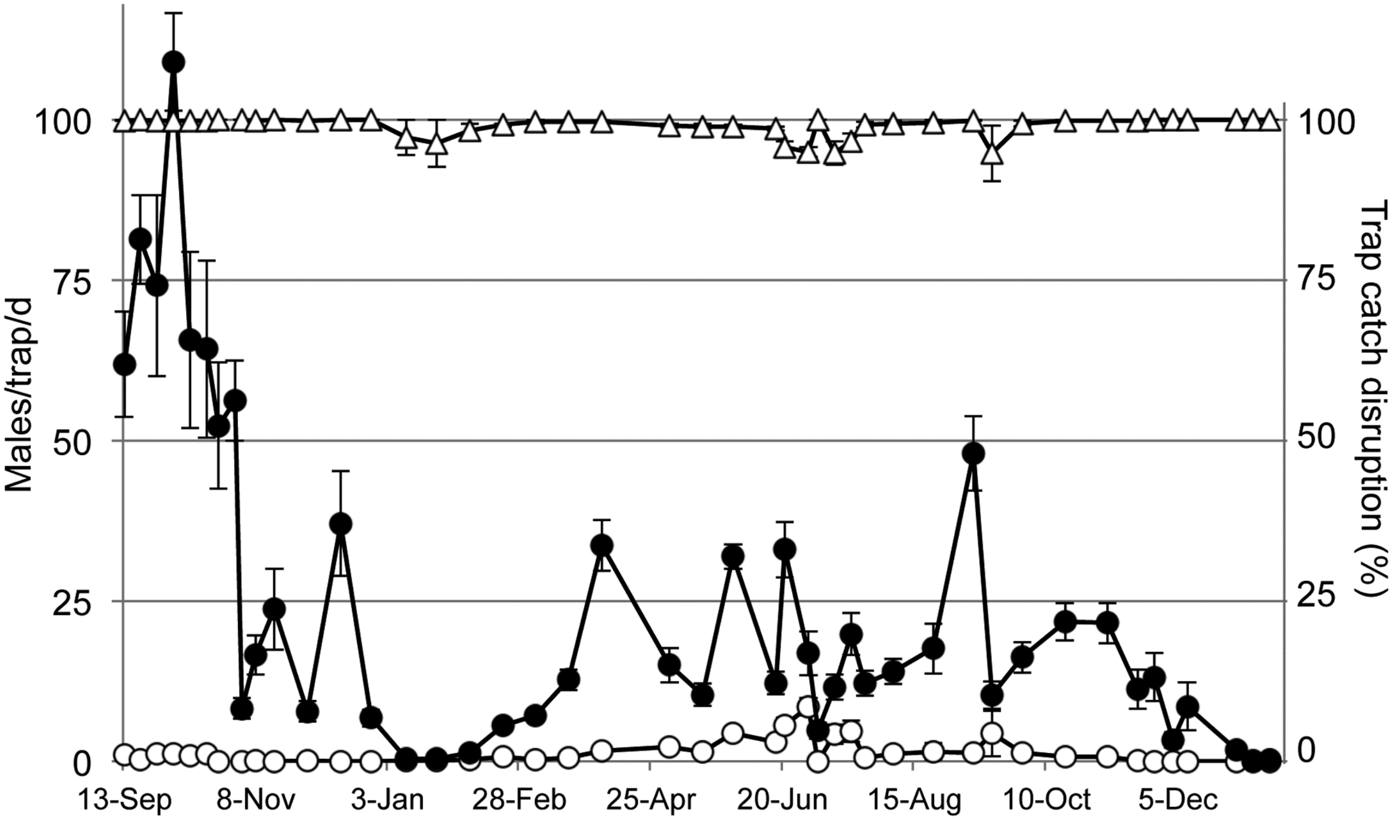

Fig. 3. Mean ± SEM (n = 14) number of male *Phyllocnistis citrella* captured in pheromone-baited traps in untreated control plots (filled circles, n = 12) and in pheromone-treated plots (open circles) of grapefruit at TRB grove, Charlotte County, Florida, USA (n = 6). Triangles represent mean ± SEM (n = 6) percentage trap catch disruption (right y axis).

means for dispenser height were compared within trap height values and means for trap height were compared within dispenser height values (Table 1).

In plots where dispensers were placed high in the canopy (≥3 m), there was no difference in trap catch disruption between the 3 trap height locations (F = 2.6216; df = 2, 81; Pr > F = 0.0788). Where dispensers were placed at the intermediate height (2 m), there was a significant effect of trap height (F = 35.6143; df = 2, 81; Pr > F < 0.0001) with less disruption occurring in traps placed at the high position (α = 0.05, Tukey's HSD). Where dispensers were placed at the lowest position (≤ 0.6 m), there was a significant effect of trap height (F = 52.3799; df = 2, 81; Pr > F < 0.0001) with significant separation of all 3 means (α = 0.05, Tukey's HSD). The least disruption occurred in traps placed high in the canopy, and the greatest disruption was observed at the lowest trap location (Table 1).

Table 1. Effect of trap and pheromone dispenser (DCEPT CLM) height on mean (± SEM) percentage trap catch disruption (TCD) of the leafminer *Phyllocnistis citrella* in mature citrus trees.

	Dispenser height[a]		
Trap ht	High	Medium	Low
High	85.5 ± 2.8 aA	86.8 ± 1.1 aA	79.0 ± 2.0 aA
Medium	83.7 ± 2.8 aA	97.0 ± 1.1 bB	95.1 ± 2.0b B
Low	91.0 ± 2.8 aA	98.4 ± 1.1 bB	99.0 ± 2.0 cB
	Main effect (dispenser height)[b]		
	86.7 ± 2.2 a	94.1 ± 3.7 b	91.0 + 6.1 b

Both main effects of the 3 × 3 factorial design and the interaction term were statistically significant (Pr > F < 0.0001). Comparisons of mean TCD values are presented for trap height within dispenser position (lower case letters) and dispenser height within trap position (upper case letters). The main effect of dispenser height (mean TCD for DCEPT CLM position across all 3 trap positions) is also presented for discussion.

[a]Means (n = 21) within columns followed by the same lower case letter and means (n = 21) within rows followed by the same upper case letter do not differ by Tukey's HSD test (α = 0.05).

[b]Main effect means (n = 63) for pheromone dispenser height followed by the same letter do not differ by Tukey's HSD test (α = 0.05).

In plots where traps were placed in the canopy tops (≥3 m), there was no significant effect of dispenser height on trap catch disruption (F = 2.6594; df = 2, 81; Pr > F = 0.0761). Where traps were placed at the intermediate height (2 m), there was a significant effect of dispenser height (F = 12.0803; df = 2, 81; Pr > F < 0.0001) with less disruption observed where dispensers were placed high in the canopy compared with the other 2 dispenser locations (α = 0.05, Tukey's HSD) (Table 1). Where traps were placed low in the canopy (≤ 0.6 m), there was a significant effect of dispenser height (F = 15.2084; df = 2, 81; Pr > F < 0.0001), and less disruption occurred where dispensers were placed high in the canopy compared with the other 2 locations (α = 0.05, Tukey's HSD) (Table 1).

For practical reasons, it may be reasonable based on subject matter interest to ignore the interaction term and examine the main effect of location of pheromone delivery device across all trap locations. The highest mean (± SEM, n = 3) trap catch disruption summed over all 3 trap locations was observed in plots where the dispensers were placed either at the intermediate (94.1 ± 3.7%) or low height (91.0 ± 6.1%) compared with plots where the dispenser was placed in the canopy tops (86.7 ± 2.2%) (α = 0.05, Tukey's HSD; Table 1).

Mean daily temperature varied only minimally with height. Mean nighttime temperatures corresponding to the period of greatest flight activity of *P. citrella* (Stelinski & Rogers 2008) were similar at the 3 heights recorded (0.6, 2.0, and 10.0 m) and averaged 17 °C during the period of 23 to 30 Oct 2013. Wind speed at night (22:00–06:00) was approximately half that of day (09:00–17:00) (Table 2).

DISSIPATION OF PHEROMONE

The temporal profile of emission of (*Z,Z,E*)-7,11,13-hexadecatrienal from DCEPT CLM dispensers fit an exponential curve (R^2 = 0.99) that approached 90% release of the initial pheromone content at 120 d after deployment in the field (Fig. 4). Data from Stelinski et al. (2010) demonstrated initial high rates of pheromone emission from the SPLAT matrix that occurred during the first days after deposition, followed by a relatively constant release rate in the order of 10 to 30 µg of pheromone per day (Fig. 4). The release rate from the dispensers was similar to SPLAT but without the initial high release seen with the SPLAT.

Discussion

The efficacy of trap catch disruption was excellent in the 3 field trials and compares very favorably with the longevity of disruption previously reported for SPLAT CLM (Stelinski & Rogers 2008). The ability of the pheromone blend or triene-only formulation to disrupt trap catch of males in traps baited with the "natural" 3:1 blend agrees closely with what we have observed and reported previously for SPLAT-based formulations (Stelinski et al. 2010; Lapointe & Stelinski 2011; Lapointe et al. 2011). Although a direct comparison of the SPLAT-based product was not made in these trials, the longevity of disruption provided by the solid dispensers far exceeded previous results obtained with SPLAT CLM™ and raises the possibility that a single application of emitters may provide year-long disruption of mating for *P. citrella*. Longevity in these studies may have been higher due to cooler temperatures during Florida's winter compared with what might be expected from deployment during the spring for disruption during the summer. Future studies are planned to apply DCEPT CLM™ to large groves (approx. 400 ha each) in the spring (Mar–Apr).

Lapointe et al. (2009) first suggested that disruption, in this species, was largely or exclusively a function of the amount of (*Z,Z,E*)-7,11,13-hexadecatrienal and that the off-ratio blend of the triene only would be superior to the "natural" 3:1 blend. The field experiment reported here upheld those predictions. Dispensers loaded with the triene component performed equally well regardless of the presence of (*Z,Z*)-7,11-hexadecadienal. The 2 formulations tested contained the same amount of triene; the addition of the diene component did not increase disruption. We conclude that inclusion of the diene in mating disruption products for this species has no benefit that would justify the additional expense of its synthesis and formulation. Also, the fact that highly effective trap catch disruption was obtained with an off-ratio blend supports the conclusion that disruption in this species falls into the category of non-competitive mechanisms (Stelinski et al. 2008) because the triene alone is not attractive to males (Lapointe et al. 2009).

The height of traps did not affect the number of males caught in the traps suggesting that males were active throughout the canopy, similar to the activity of males of the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) (Girling et al. 2013). But the location of *P. citrella* pheromone dispensers in the tree canopy did have a significant effect on trap catch disruption. Dispensers placed low in the canopy (< 0.6 m) resulted in decreased disruption in the center and top of the canopy compared with disruption achieved low in the canopy. Similarly, dispensers located in the canopy center (2 m) were more effective at disruption of traps located low or in the center of the canopy; less disruption was achieved in the canopy top. These results suggest that the net displacement of the pheromone was downward under our conditions, perhaps because the triene (molecular weight 234) is heavier than air and would tend to settle under calmer conditions that prevail in mature citrus orchards in Florida at the time of greatest flight activity by males (Stelinski & Rogers 2008). Schal (1982) showed that odor plumes in tropical forests can rise with convective currents, and Girling et al. (2013) observed net upward movement of the plume from pheromone dispensers for disruption of *A. transitella*. Temperature conditions at our location may not have been favorable for upward movement of the pheromone. Our results also showed that dispensers located high in the canopy were equally effective at disruption of traps at all 3 heights, but less effective at disrupting traps located low and in the center of the canopy compared with dispensers located low and in the center of the canopy. A possible explanation of this phenomenon is that air movement above the canopy dispersed or disrupted the pheromone plume and therefore decreased the amount of pheromone entering the tree canopy compared with dispensers located within the canopy.

Because of the high level and longevity of trap catch disruption we observed, it may be possible to reduce the coverage and therefore the

Table 2. Meteorological data for the period of 23 to 30 October 2013, western St. Lucie County, Florida, USA.

	Temperature (°C)								
				Night			Day		
	24 h (*n* = 192)			22:00–06:00, *n* = 72			09:00–17:00, *n* = 65		
Ht (m)	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
0.6	30.0	13.0	21.4 ± 0.4	21.4	13.0	16.5 ± 0.2	30.0	21.9	26.8 ± 0.2
2.0	29.6	14.1	21.7 ± 0.3	21.8	14.1	17.1 ± 0.2	29.6	22.0	26.8 ± 0.2
10.0	27.7	15.1	21.3 ± 0.3	21.8	15.1	17.4 ± 0.2	27.7	21.1	25.5 ± 0.2

	Wind speed (km/h)		
	24h	Day	Night
Min (Max)	1.9 (20.8)	5.3 (20.8)	1.9 (14.0)
Mean + SEM (*n*)	9.8 ± 0.35 (192)	14.3 ± 0.50 (72)	6.8 ± 0.34 (65)

Source: Florida Automated Weather Network (fawn.ifas.ufl.edu)

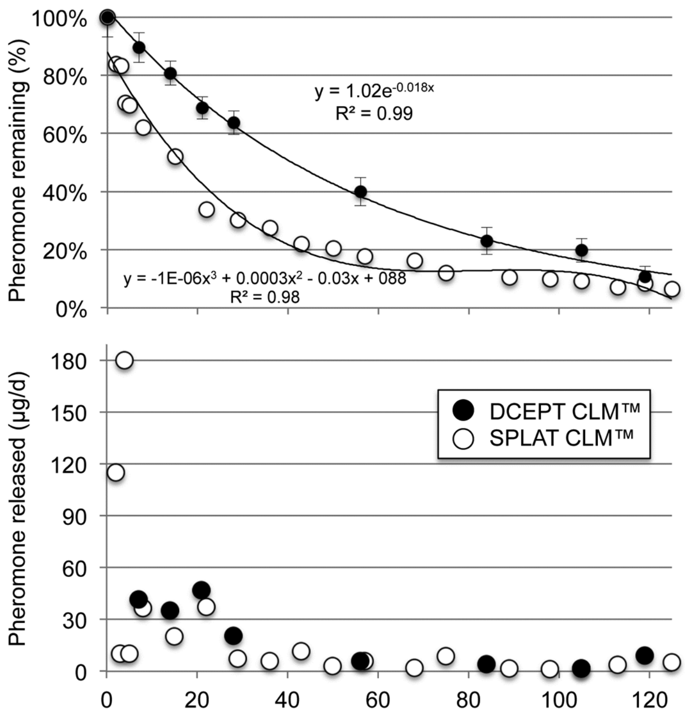

Fig. 4. Pheromone release profiles for DCEPT CLM™ (closed circles) and SPLAT CLM™ (open circles). DCEPT CLM data (top graph) are mean (± SD) percentage of initial amount of (*Z,Z,E*)-7,11,13-hexadecatrienal remaining in the dispensers (*n* = 10). SPLAT CLM points are equivalent data previously published (Stelinski et al. 2010). The amount of pheromone released (bottom graph) was calculated as the mean difference in pheromone remaining from the preceding period.

cost of disruption using DCEPT CLM in a manner similar to that proposed for SPLAT CLM (Lapointe et al. 2014) and as demonstrated for gypsy moth disruption (Tcheslavskaia et al. 2005). The inclusion of skip rows or other deployment patterns should be explored to optimize cost and efficacy. The cost of the required manual labor for deployment of solid dispensers hung from the tree branches would appear to be offset by the longevity of the solid dispenser. It now appears possible to achieve season-long disruption of *P. citrella* with a single deployment of a pheromone dispenser. This would be a major advance in the control of this leafminer and associated citrus canker disease, and a major contribution to the environmentally appropriate control of a major citrus pest.

The release profile of DCEPT CLM may contribute to greater longevity in the field compared with a SPLAT product. SPLAT is a flowable paste-like material that requires time (hours) to dry sufficiently to be rainfast and days to acquire a "skin" that retards the rate of pheromone release. As a result, the emission profile for (*Z,Z,E*)-7,11,13-hexadecatrienal from SPLAT showed a higher rate over the first ~20 d (Fig. 4B) compared with a subsequent prolonged period of release of ~10 µg/d. In contrast, the emission profile of DCEPT CLM displayed classic first-order kinetics with a half-life of ~40 d calculated from the exponential equation displayed in Fig. 4, and a sustained rate of release greater than that of SPLAT for ≤100 d.

We speculate, based on personal observations, that the current intensive and unsustainably high use of broad-spectrum insecticides in Florida to control the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), and citrus greening disease have reduced or eliminated natural and introduced biological control of *P. citrella* such as the parasitoid *Ageniaspis citricola* Lonvinovskaya (Hymenoptera: Encyrtidae) resulting in abnormally high leafminer populations throughout the state. Trap catch of *P. citrella* in untreated locations often exceeded 100 males per trap per day despite grower insecticide applications. If this situation changes, we feel that reestablishment of biological control of *P. citrella* should be a high priority. The combination of biological control and mating disruption of *P. citrella* will be a major contribution to integrated control of this important pest and citrus canker disease.

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