Season-long mating disruption of citrus leafminer, *Phyllocnistis citrella* Stainton, with an emulsified wax formulation of pheromone

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Abstract

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a major worldwide pest of citrus. Larval feeding by this insect facilitates proliferation of citrus bacterial canker, *Xanthomonas axonopodis* pv. citri. Herein, we describe a season-long disruption trial of *P. citrella* with a newly developed, emulsified wax dispenser of pheromone (SPLAT-CLM^{TM}). A formulation containing a 3 : 1 blend of (*Z,Z,E*)-7,11,13-hexadecatrienal:(*Z,Z*)-7,11-hexadecadienal at a 0.2% loading rate of active ingredient by weight and deployed twice per season (24 weeks total) at 490 g of formulation/ha caused season-long disruption of male moth catch in pheromone traps as well as reduced leaf infestation. Analysis of pheromone release from dispensers by gas chromatography revealed that effective disruption of *P. citrella* occurred at a deployment rate of 126 μg of (*Z,Z,E*)-7,11,13-hexadecatrienal/ha/h. Direct observation of moth behaviour in the field suggested that disruption by this formulation occurred by a non-competitive mechanism. A formulation of the 3 : 1 attractive blend at a 0.02% pheromone loading rate caused only 2–6 weeks of disruption per deployment and did not reduce leaf infestation during mid and end of the season evaluations. A formulation containing 0.2% of (*Z,Z*)-7,11-hexadecadienal alone and deployed at 490 g/ha caused 6–7 weeks of moth disruption to pheromone traps and did not prevent leaf infestation, while an identical formulation loaded with 0.02% (w/w) of (*Z,Z*)-7,11-hexadecadienal alone had no effect on *P. citrella* orientation to pheromone traps. The SPLAT formulation evaluated herein appears to be an excellent release device for (*Z,Z,E*)-7,11,13-hexadecatrienal given that approximately 100 days of steady release occurred following an initial brief (ca. 7 days) burst of higher release. The advantages of SPLAT as a formulation for *P. citrella* disruption include low cost of manufacturing, biodegradable and weather resistant characteristics, and flowability allowing machine application. Mating disruption should be an effective alternative to insecticides for management of *P. citrella* and may reduce the incidence of citrus canker.

Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is an important pest of commercial citrus production throughout the world (Heppner 1993). Larvae damage leaves by creating serpentine feeding mines, which have been shown to reduce yield (Peña et al. 2000). More importantly,

leaf wounds caused by larval feeding predisposes trees to infection by citrus bacterial canker, *Xanthomonas axonopodis* pv. citri, which results in blemished fruit, premature fruit drop, leaf drop, and tree decline (Graham et al. 2004). Feeding *P. citrella* larvae wound the leaf cuticle, which exposes leaf mesophyll to direct infection. Wounds caused by *P. citrella* do not heal readily, which further increases the exposure period to the bacterium and its spread by mobile larvae throughout feeding galleries (Graham et al. 2004). *Phyllocnistis citrella* is a pest of all citrus varieties as well as other Rutaceae and certain ornamentals; however, grapefruit, tangerine, and pumello are the most susceptible hosts (Legaspi and French 2003).

Control of larval *P. citrella* with insecticides is inconsistent and at times ineffective because larvae within the leaf mines are protected from foliar spray residues. Typical spray programs may require bi-weekly insecticide applications to protect emerging, highly susceptible leaf flush. Such programs also reduce the populations of natural enemies of *P. citrella* and other citrus pests (Peña et al. 2002). Development of effective alternatives to conventional insecticides for *P. citrella* management is of critical importance for leading citrus-producing countries, including Brazil and the USA, where citrus canker is known to limit yield and profit (Leite and Mohan 1990).

The complete sex pheromone blend of *P. citrella* was recently reported (Leal et al. 2006; Moreira et al. 2006). Of three total components identified, a 3 : 1 blend of (Z,Z,E)-7,11,13-hexadecatrienal and (Z,Z)-7,11-hexadecadienal is sufficient to optimally attract males to pheromone traps (Leal et al. 2006; Moreira et al. 2006); however, neither single component is attractive by itself (Lapointe et al. 2009). Following identification of the pheromone, it was confirmed that it is highly attractive to male *P. citrella* in Florida, USA (Lapointe et al. 2006). Monitoring protocols with pheromone traps were optimized soon thereafter (Stelinski and Rogers 2008). Furthermore, mating disruption of *P. citrella* has been investigated in both Japan (Mafi et al. 2005) and the USA (Stelinski et al. 2008).

Control of *P. citrella* by mating disruption has proven highly effective at a remarkably low deployment rate of pheromone [1.5 g of active ingredient (AI)/ha] (Stelinski et al. 2008). However, the high cost of synthesis for the *P. citrella* pheromone components limits the commercial viability of mating disruption for this pest even though very little pheromone is needed to achieve efficacy. Given the species-specific mode of action of mating disruption, *P. citrella* management may be constrained due to the emergence of *Diaphorina citri* Kuwayama as another global pest currently threatening the sustainability of citriculture (Halbert and Manjunath 2004). *Diaphorina citri* vectors phloem-restricted bacteria in the genus *Candidatus* Liberibacter that cause huanglongbing disease, which kills citrus trees (Bové 2006). Therefore, both effective and economical behavioural modification tools are needed for management of *P. citrella*.

One potential option for more economical mating disruption for *P. citrella* is development of a single pheromone component release device. This option is feasible because mating disruption of *P. citrella* occurs by a non-competitive mechanism; i.e., camouflage or desensitization (Stelinski et al. 2008; Lapointe et al. 2009), and because both individual components can disrupt male orientation to the complete two-component attractive blend (Lapointe et al. 2009). Given that (Z,Z)-7,11-hexadecadienal is much less expensive to synthesize than (Z,Z,E)-7,11,13-hexadecatrienal (Lapointe et al. 2009), we sought to determine whether disruption of *P. citrella* could be achieved by deploying this single component in an effort to reduce cost of mating disruption for this pest. Furthermore, although disruption of *P. citrella* has proven effective with hand-applied rubber devices (Stelinski et al. 2008), a flowable formulation amendable to machine application (Stelinski et al. 2007) is required if mating disruption is to be adopted on large (>5000 ha) citrus growing operations, which occur in the USA and Brazil. Given that commercially produced citrus is grown on such large scales, hand application of pheromone dispensers would be economically prohibitive.

The goal of this investigation was to develop a flowable emulsified wax formulation for pheromone release amendable to mechanical application for large scale mating disruption of *P. citrella*. The specific objectives of this investigation were to: (i) compare the effect of the attractive two-component *P. citrella* pheromone blend vs. a single pheromone component when released from emulsified wax on disruption of male moth orientation and crop injury; (ii) evaluate two loading rates of each pheromone treatment; (iii) gain insights into the mechanism of disruption by direct observation of moth behaviour in the field; and (iv) quantify season-long pheromone release rate by gas chromatography in order to relate efficacy data with seasonal pheromone release.

Materials and Methods

Pheromone release formulation

A flowable formulation of emulsified wax designed to provide slow release of semiochemicals (SPLAT^{TM}; ISCA Technologies, Riverside, CA) was formulated with either (Z,Z,E)-7,11,13-hexadecatrienal and (Z,Z)-7,11-hexadecadienal (henceforth referred to as triene and diene, respectively) or the diene alone, each at two loading dosages. Both pheromone components were synthesized by ISCA Technologies Inc., as previously described (Leal et al. 2006; Moreira et al. 2006). The triene was 94% and 90% chemically and isomerically pure, respectively, while the diene was 86% and 72% chemically and isomerically pure, respectively. The diene was chosen as the single component for evaluation because *P. citrella* disruption occurs via a non-competitive mechanism (Stelinski et al. 2008) and can be achieved with either the triene or diene (Lapointe et al. 2009); however, the diene is approximately 10-fold less expensive to synthesize than the triene (Lapointe et al. 2009). Each pheromone blend formulation was incorporated into SPLAT at 0.2% or 0.02% AI by weight. This resulted in a total of four pheromone treatments for field testing.

Field plots and experimental design

The experiment was conducted in a 9-year-old 40.5-ha orange orchard [(Citrus sinensis [L.]) var 'Valencia.'] near Orlando, FL, USA. Trees were planted on a 3×6 m spacing and average canopy height was 4 m. The orchard was managed by the University of Florida according to commercial pruning, irrigation, herbicide, and fungicide management practices but without insecticides. Disruption trials were conducted comparing five treatments: (i) untreated control; (ii) 3 : 1 triene:diene blend at a 0.2% AI loading; (iii) 3 : 1 triene:diene blend at a 0.02% AI loading; (iv) diene alone at 0.2% AI loading; and (v) diene alone at 0.02% AI loading. The experiment was arranged as a randomized complete block design with five replicates, each replicate consisting of approximately 100 trees (ca. 0.25 ha). Replicate plots were separated by 40 m and blocks of treatments were separated by 50 m. SPLAT treatments were applied using custom made hand-held applicators calibrated to deliver 1.0 g dollops. Point source density and concentration of pheromone tested were based on previously optimized disruption reported with the 3 : 1 triene:diene blend released from rubber septum dispensers (Stelinski et al. 2008). Each tree within a treatment plot received approximately 3 dollops of SPLAT resulting in approximately 490 g of each formulation per ha. SPLAT was applied to tree branches within canopies approximately 2.0 m above ground level, which is the location of greatest male *P. citrella* activity within trees approximately 4 m tall (Stelinski and Rogers 2008). The experiment was divided into two 12-week intervals comprising (i) spring/early summer and (ii) late summer/fall. Treatment reapplication was deemed necessary at 12 weeks into the trial based on a recent investigation of *P. citrella* disruption with a rubber sleeve dispenser of pheromone (Stelinski et al. 2008) and because disruption of trap catch in some treatments had declined to levels comparable to controls (see Results). Treatments were applied on 1 May 2008 and 23 July 2008; disruption of *P. citrella* flight and associated damage by larvae were evaluated over the course of an entire growing season.

Treatment evaluation

Disruption of male *P. citrella* orientation was quantified using two pheromone traps (LPD Scenturion Guardpost; Suterra, Bend, OR) deployed within each replicate plot. One trap was placed in the central tree of each plot and the second was placed two rows from the plot edge. All traps were baited with a single red rubber septum lure loaded with 0.1 mg of triene and 0.03 mg of diene as this has been shown to be highly effective for trapping male *P. citrella* in Florida (Lapointe et al. 2006; Stelinski and Rogers 2008). Monitoring traps were hung at least 1.0 m from the nearest mating disruption dispenser, at approximately 1.5–2 m above ground level in mid-canopy (Stelinski and Rogers 2008). Pheromone lures were replaced approximately every 6 weeks throughout the season based on known longevity of attractiveness (Lapointe and Leal 2007). Adhesive trap liners were replaced and counted weekly.

In addition to monitoring disruption by quantifying male catch in pheromone traps, damage to newly flushed leaves was assessed at the end of two 12-week treatment deployment intervals on 23 July and 10 October 2008. Twenty shoots, 10 from mid-canopy (2.5 m) and 10 from lower canopy (1.0 m), were inspected at random from 20 trees per replicate block (2000 flush samples per treatment) and the number of shoots per tree containing live mining *P. citrella* larvae was recorded.

Behavioural observations in the field

SPLAT droplets from each of the four formulation treatments were directly observed in the field to test the hypothesis that male *P. citrella* approach pheromone dispensers. Previous investigations have confirmed that male moths of various species orient to, closely approach, and even contact pheromone dispensers of a wide range of release rates in the field, which suggests that competitive attraction is an operative mechanism of disruption for these species (Stelinski et al. 2004; Epstein et al. 2006). Observations of dispensers in tree canopies were conducted for approximately 2 h each night between 21:00 and 23:00 hours, the time when male *P. citrella* are known to respond to pheromone lures in the field (Stelinski and Rogers 2008). Observations were conducted on 10 nights between 5 May and 1 October 2008 (5, 20 May; 6, 13 June; 8, 14 July; 15, 28 August; 12 September; 1 October). An observer rotated among plots conducting 20-min observational bouts per treatment such that multiple treatments were observed on a given night. The order of observations across treatments was randomized nightly and different sets of dispensers were observed on each night.

In addition, male moth orientation to pheromone traps (as described above) was observed for 2-h periods during the same interval in control plots to verify that male *P. citrella* could be observed orienting to an attractive point source in the field under the same conditions during which SPLAT dispensers were being observed. The number of moths observed orienting to traps and the number caught in traps under observation were assessed. In observations of both dispensers and traps, data were dictated into a hand-held microcassette audio recorder by an investigator standing 0.25–0.5 m from the pheromone source under observation. Observations employed night-vision goggles (Rigel, Model 3250, DeWitt, IA) as described by Stelinski et al. (2004).

Release rate measurements

Samples of the 3 : 1 triene:diene blend formulation of SPLAT at the 0.2% pheromone loading rate were applied as 1.0 g dollops to 2×3 cm acetate film strips. The strips were stapled individually to five 5×45 cm wooden boards with wires attached at each corner. One sample was taken from each board at test onset and prior to field aging and placed in a pre-cleaned (acetonitrile rinse) 20-ml scintillation vial (Fisher Scientific, Pittsburgh, PA). Five millilitres

of an internal standard solution of 104 ng/μl of undecanal in acetonitrile (99.9% purity; Sigma-Aldrich, St. Louis, MO) was added to each vial. These samples were placed at $-10°C$ until use for subsequent analyses. The boards containing the remaining samples were maintained at 24°C in the laboratory for 24 h and then placed in the field on 22 May 2008. The boards were hung horizontally within the canopies of five 4-m-tall trees in a mixed citrus planting at the Citrus Research and Education Center (Lake Alfred, FL, USA). The boards were hung approximately 1.5 m above ground on the edges of trees in partial shade created by higher branches. Samples were collected from all boards 1, 2, 3, 4, and 7 days following deployment in the field and then weekly for a total of 15 weeks. All samples were transported to the laboratory individually within scintillation vials with 5 ml of the internal standard solution described above. All samples were maintained frozen as described above prior to analyses.

Immediately before analysis, each sample was removed from the freezer and placed in a water bath at 80–85°C for 3–5 min. Thereafter, vials were shaken for 1 min and returned to the water bath for 3 min, then removed and agitated again. The solvent was removed from each sample with a glass Pasteur pipette and filtered through a second pipette, fitted with a $4.5 \times 6.5 \times 8$ cm triangular piece of Kimwipe (Kimberly-Clarke Corp., Roswell, GA) folded to form a plug at the tapered end of the pipette, into a pre-cleaned 8-ml vial with a polytetrafluoroethylene-lined cap (Chromatography Research Supplies Inc., Louisville, KY).

Pheromone within samples was quantified using a Varian CP3800 gas-chromatograph (GC) (Varian Inc., Palo Alto, CA) fitted with a 30-m Rtx-5 capillary column (0.25 mm I.D.; 0.5 μm film) (Restek Corp., Bellefonte, PA) and equipped with a flame-ionization detector. The initial GC temperature was held at 50°C for 1 min and then increased at a rate of 10°C/min to a final temperature of 260°C where it was held for 1 min. The carrier gas was He at a flow rate of 1 ml/min. One microlitre of each sample vial was injected for analysis. The pheromone content of the samples was calculated using the internal standard method (McNair and Miller 1998) and was corrected for the original weight of each sample.

Statistical analyses and other calculations

Moth catch data were transformed to ln $(x + 1)$ to normalize distribution and homogenize variance and then subjected to analysis of variance (ANOVA). Per

cent injury data from the two sampling dates were arcsine transformed prior to ANOVA. Means were separated by Tukey's test (SAS Institute 2000) following significant ANOVAS (α = 0.05). Percent orientational disruption of male moth catch in traps was calculated as 1 − (mean moth catch per trap in the pheromone-treated plot/mean moth catch per trap in the control plot) × 100.

Results

Disruption of male *P. citrella* catch in pheromone traps

During the 12-week period following the first SPLAT application, disruption of male *P. citrella* catch in pheromone traps in plots treated with 0.2% 3 : 1 blend was consistently around 90%, relative to control plots (fig. 1a). Moth catch in traps within plots treated with 0.02% 3 : 1 blend was reduced only during the first 2 weeks compared with untreated plots (fig. 1a). During the initial 7 weeks of the early season trial, disruption of male trap catch in plots treated with 0.2% diene ranged from 50% to 90%;
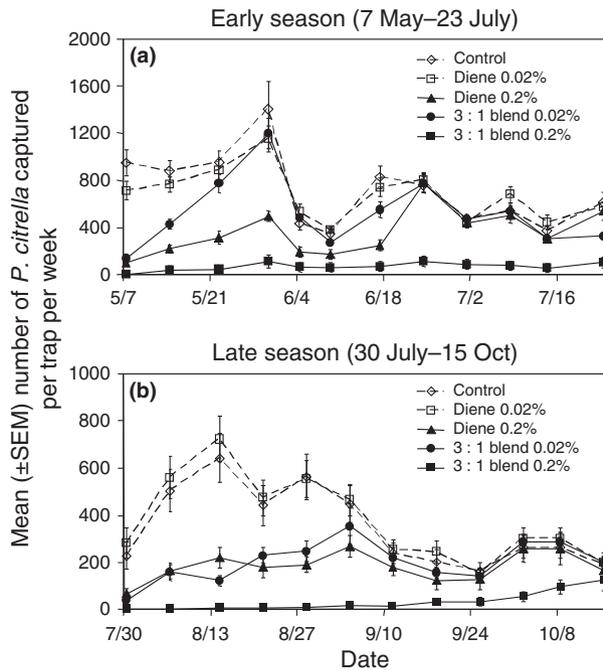
by the eighth week there was no longer a discernable effect of this treatment relative to the control (fig. 1a). Weekly male catch in plots treated with 0.02% diene was approximately equivalent to that observed in control plots (fig. 1a). Over the course of the initial 12 weeks of the investigation, significantly (F = 12.3; d.f. = 4, 16; P < 0.001) fewer male *P. citrella* were captured in plots treated with 0.2% diene and 3 : 1 blend than in control plots (fig. 2a). However, significantly (P < 0.001) fewer males were captured in plots treated with 0.2% 3 : 1 blend compared with 0.2% diene (fig. 2a). At the 0.02% loading dosage, neither pheromone treatment formulation reduced moth catch in traps compared with the control (P values > 0.05) (fig. 2a).

During the 12-week period following the second SPLAT application (late summer/fall), *P. citrella* population densities were generally lower compared with the period following the first SPLAT application (spring/early summer) (fig. 1b). Disruption of male catch in plots treated with 0.2% 3 : 1 blend averaged approximately 90% but tended to decrease near the end of the trial (fig. 1b). Disruption following the second SPLAT application with 0.2% diene lasted 6 weeks, similar to that observed during spring and early summer (fig. 1b). Disruption of 40–80% was
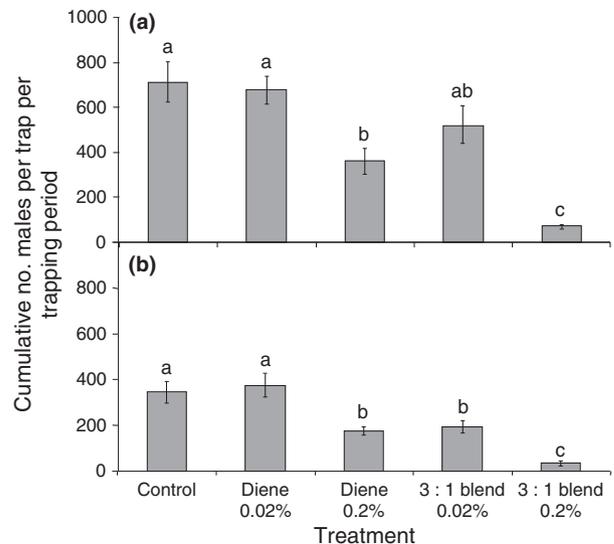

Fig. 1 Mean ± SEM captures of *Phyllocnistis citrella* males per week in the spring and early summer (a) and late summer and fall (b) throughout the 2008 season in replicated 0.25-ha pheromone-treated and control plots. Pheromone treatments were applied on 1 May and 23 July. 3 : 1 Blend refers to a 3 : 1 blend of (Z,Z,E)-7,11,13-hexadecatrienal and (Z,Z)-7,11-hexadecadienal. Diene refers to (Z,Z)-7,11-hexadecadienal.

Fig. 2 Mean ± SEM captures of male *Phyllocnistis citrella* in pheromone-baited traps in plots treated with various formulations of SPLAT pheromone dispensers over the course of two 12-week periods during early (a) and late (b) season. 3 : 1 Blend refers to a 3 : 1 blend of (Z,Z,E)-7,11,13-hexadecatrienal and (Z,Z)-7,11-hexadecadienal. Diene refers to (Z,Z)-7,11-hexadecadienal. Bars within each panel followed by the same letter are not significantly different (α = 0.05, ANOVA followed by Tukey's test).

observed with 0.02% 3 : 1 blend for the initial 4 weeks following the second SPLAT application (fig. 1b), which was approximately twice as long as that observed during the early season interval (fig. 1a). Consistent with the first part of the season, there was no evidence of disruption with 0.02% diene throughout this second deployment interval (fig. 1b). Over the course of the entire second deployment interval, significantly (F = 8.9; d.f. = 4, 16; P < 0.001) fewer male *P. citrella* were captured in plots treated with 0.2% diene as well as in those treated with the 3 : 1 blend at both loading dosages compared with control plots (fig. 2b). However, significantly (P < 0.001) fewer males were captured in plots treated with 0.2% 3 : 1 blend than in any other treatment (fig. 2b).

Leaf infestation by larval *P. citrella*

Following the initial 12 weeks of the study, significantly (F = 7.4; d.f. = 4, 16; P < 0.001) fewer flush shoots were infested with larval *P. citrella* in plots treated with 0.2% 3 : 1 blend compared with control plots (fig. 3a). There was no significant (P val-

ues > 0.05) difference in injury between any of the other treatments and the control (fig. 3a).

At the end of the study, significantly (F = 6.4; d.f. = 4, 16; P = 0.005) less leaf injury was recorded in plots treated with 0.2% 3 : 1 blend compared with control plots (fig. 3b). There was no difference (P values > 0.05) in injury between any of the other treatments tested compared with the control (fig. 3b).

Behavioural observations in the field

No male *P. citrella* were observed orienting to SPLAT dollops in plots treated with any of the pheromone formulations evaluated during 20 h of observation over 10 days of sampling. In contrast, 34.0 ± 2.1 (mean ± SEM) males/night were observed orienting to monitoring traps baited with a 3 : 1 lure (*N* = 10 nights) in control plots. Of those moths observed, 19.6 ± 0.9 male *P. citrella* were captured/trap per night on average.

Release rate measurements

The release profile of triene from SPLAT over the course of the season, shown in fig. 4, fits a third order polynomial decay curve at an $R^2 = 0.98$. During the initial 7 days of measurement, release rate of triene occurred at approximately 70 μg/day (fig. 4a). However, for the remaining 104 days of measurement, the release rate appeared relatively linear at approximately 6.3 μg of triene/day (fig. 4a). During this latter 104 days of release, pheromone loss per day from SPLAT dollops remained at a constant decay of approximately 0.5–4.0% (fig. 4b).

Discussion

Of the SPLAT treatments evaluated, the formulation containing a 3 : 1 blend of the two-component attractive *P. citrella* blend was the only one to cause season-long disruption as well as reduce leaf infestation. However, it required deploying 490 g/ha of the SPLAT formulation containing 0.2% AI by weight twice per season, which amounts to deploying 2 g of *P. citrella* pheromone per ha per season. Although reduced *P. citrella* larval injury should contribute to reduced incidence of citrus canker infection (Graham et al. 2004), we were unable to document this hypothesis because the disease did not appear to be present in the citrus orchard where experiments were conducted (data not shown). Recently, Lapointe et al. (2009) demonstrated that disruption
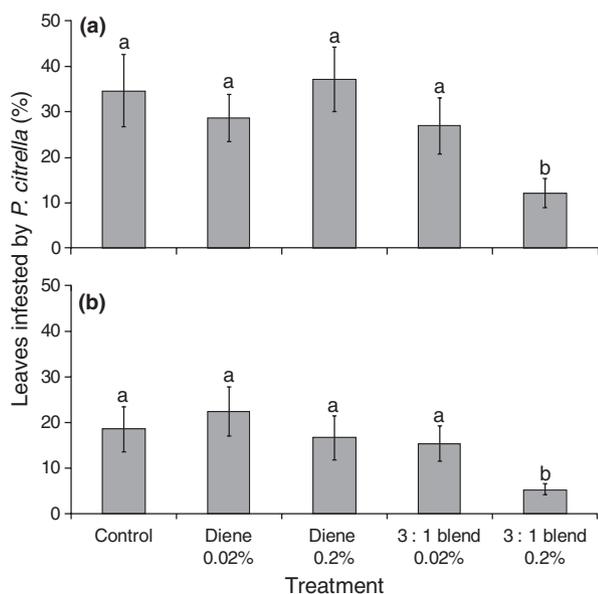

Fig. 3 Mean ± SEM percent leaf flush infestation by *Phyllocnistis citrella* larvae during early (a) and late (b) season as influenced by various blend and dosage treatments of SPLAT pheromone dispensers applied to replicate 0.25-ha plots of citrus. Evaluations were conducted at the end of two 12-week periods on 23 July and 10 October. On each date, 2000 leaf flushes were evaluated per treatment. Bars within each panel followed by the same letter are not significantly different (α = 0.05, ANOVA followed by Tukey's test).
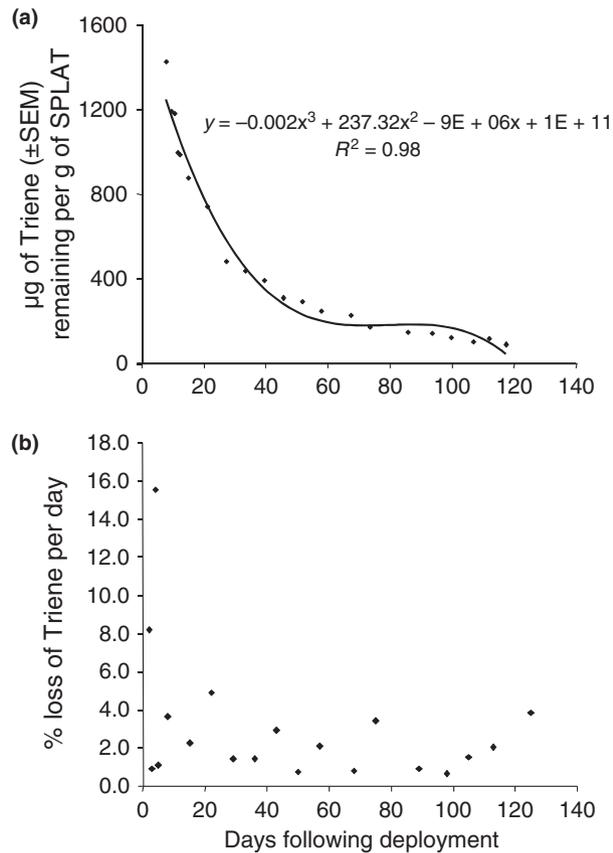
Fig. 4 Weekly release profile of (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal from 1.0 g dollops of SPLAT containing 0.2% of a 3 : 1 blend of (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal and (*Z*,*Z*)-7,11-hexadecadienal by weight.

of *P. citrella* is largely driven by the triene pheromone component; therefore, release of this component from the two 3 : 1 blends probably mediated disruption in this trial. Our release rate quantification indicated that effective disruption of *P. citrella* occurred when 1 g SPLAT dollops were releasing 0.26 µg of triene/h. Therefore, approximately 126 µg of triene/ha/h are required to effectively disrupt *P. citrella*. Male *P. citrella* did not approach dollops of SPLAT of any formulation tested in this investigation, which further confirms that disruption of this species occurs by a non-competitive mechanism (Stelinski and Rogers 2008; Lapointe et al. 2009). The amount of pheromone required for effective disruption of *P. citrella* is much lower than what is required for other species. For example, 5–20 mg AI/ha/h are required for effective disruption of the oriental fruit moth, *Grapholita molesta* (Busck); 15 mg AI/ha/h are required for lightbrown apple moth, *Epiphyas postvittana* (Walker); and 10–35 mg/ha/h are required for grape berry moth, *Paralobesia viteana*

(Clemens); several other examples are in the same range of tens of mg AI/ha/h for effective disruption (reviewed by Cardé and Minks 1995). Given that disruption of *P. citrella* did not fall below 90% with the 3 : 1 blend treatment at the 0.2% loading level, it is possible that the threshold release rate for effective disruption is below 100 µg of triene/ha/h. Therefore, the pheromone release rate required to disrupt *P. citrella* is approximately 100-fold lower than for other lepidopteran species where this has been quantified.

The SPLAT formulation appears to be an excellent release device for the *P. citrella* pheromone given that approximately 100 days of steady release occurred following an initial brief (ca. 7 days) burst of higher release (fig. 4). These results are in contrast to what has been quantified with the major pheromone components of codling moth [*Cydia pomonella* (L.)], (*E*,*E*)-8,10-dodecadien-1-ol, (Epstein et al. 2006); *G. molesta*, (*Z*)-8-dodecen-1-yl-acetate, (Stelinski et al. 2005); and *P. viteana*, (*Z*)-9-dodecenyl acetate, (Jenkins and Isaacs 2008) from SPLAT emulsified wax formulations. In each of those cases, the pheromones evaporated from emulsified wax according to first order exponential functions. The probability of insect pheromone evaporation from solid surfaces decreases as chain length of the chemical increases and may be influenced by functional groups (Gut et al. 2004). Therefore, it is possible that the seemingly linear and much lower release rate of the principal *P. citrella* pheromone component [(Z,Z,E)-7,11,13-hexadecatrienal] from SPLAT relative to the 12 carbon tortricid pheromones discussed above, is due its longer chain length. The remarkably low concentration of pheromone required for effective disruption of *P. citrella* combined with its favourable release characteristics from wax dispensers makes it an excellent candidate for control by mating disruption.

Unfortunately, our explorations to reduce cost of mating disruption formulations for *P. citrella* by either decreasing the amount of AI per g of SPLAT or releasing the diene component alone were unsuccessful. The 0.02% 3 : 1 triene:diene blend disrupted male *P. citrella* for only 2–5 weeks (fig. 1a,b) suggesting that release rate from this formulation rapidly dropped below the threshold required for effective disruption. This duration of efficacy would likely require at least six deployments of the 0.02% 3 : 1 blend formulation to maintain season-long disruption. The 0.2% diene formulation also showed some promise causing approximately 6–7 weeks of measurable disruption, but was also not sufficiently

effective to maintain season-long disruption. The first investigation of *P. citrella* mating disruption reported near 100% effective orientation disruption with the diene alone; although, the small 0.08-ha test plots did not resolve a treatment effect with respect to larval infestation (Mafi et al. 2005). However, Mafi et al. (2005) deployed the diene from 500 to 1300 polyethylene tubes per ha at a loading of 60 mg of diene per tube. Although they did not quantify release rate from the polyethylene tubes, such dispensers loaded with that quantity of pheromone are known to release 10–30 mg of AI/ha/h (Witzgall et al. 2008); therefore, the release rate of diene in their study may have been at least an order of magnitude higher than in the current investigation. Furthermore, Lapointe et al. (2009) recently provided evidence that a 2% diene only SPLAT formulation may be approximately equivalent to the 0.2% 3 : 1 blend SPLAT formulation tested in this study; however, their trial was conducted for only 26 days in late season (October). The above results suggest that effective season-long disruption of *P. citrella* should be possible with the diene component alone. In future studies, we plan on testing the efficacy of diene formulations at deployment rates higher than those tested herein with season-long trials. The threshold release rate for effective disruption of *P. citrella* with the diene component remains to be determined. Given that the diene comprises only a third of the 2-component attractive *P. citrella* blend, it is perhaps not surprising that more diene than triene should be required for effective disruption of *P. citrella* by a non-competitive mechanism such as desensitization or sensory imbalance (Bartell 1982).

The emulsified wax dispenser for semiochemical release was developed over a decade ago (Atterholt et al. 1998). Since then, effective formulations have been developed, and in some cases are now commercially available, for several lepidopteran pests including *C. pomonella* (Epstein et al. 2006), *G. molesta* (Stelinski et al. 2007), *P. viteana* (Jenkins and Isaacs 2008) the gypsy moth, *Lymantria dispar* (L.), (Tcheslavskaia et al. 2008) among others. Recently, an attract & kill version of an emulsified wax formulation was also developed for control of a dipteran, the oriental fruit fly [*Bactrocera dorsalis* (Hendel)] (Vargas et al. 2009). Emulsified wax formulations, such as SPLAT, for release of semiochemicals offer several advantages over more traditional hand-applied reservoir dispensers. They are inexpensive to manufacture and store, biodegradable, relatively weatherproof, and amendable to machine application by ground or air equipment. Machine application would be a requirement for *P. citrella* management by mating disruption in the main citrus-producing countries such as the USA and Brazil given that citrus is grown as multi-thousand ha monocultures. Mechanized application devices have been developed for SPLAT (Stelinski et al. 2007) and should be easy to adopt for application in commercial citrus. ISCA Technologies has initiated registration of an emulsified wax formulation for control of *P. citrella* by mating disruption (SPLAT-CLM^{TM}) in commercial citrus in the USA and Brazil. In the interim, we continue to optimize the formulation and are developing a mechanized delivery system for use in commercial citriculture.

Acknowledgements

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