Entomologia
Experimentalis et Applicata

DOI: 10.1111/j.1570-7458.2011.01178.x

# An applicator for high viscosity semiochemical products and intentional treatment gaps for mating disruption of *Phyllocnistis citrella*

S. L. Lapointe[1]* & L. L. Stelinski[2]

[1]*United States Department of Agriculture, Agricultural Research Service, United States Horticultural Research Laboratory, 2001 South Rock Road, Fort Pierce, FL 34945, USA, and* [2]*Entomology and Nematology Department, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850, USA*

Accepted: 11 August 2011

*Key words*: sex pheromone, (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal, citrus leafminer, SPLAT, Lepidoptera, Gracillariidae

## Abstract

The leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a global pest of citrus, and contributes to the incidence and severity of citrus bacterial canker, X*anthomonas axonopodis* pv. citri. SPLAT-CLM™ (ISCA Technologies) is an emulsified wax product that provides sustained release of (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal, the major component of the *P. citrella* sex pheromone. Here, we report success in development of a mechanized and automated applicator of SPLAT and other high viscosity products on a large scale in tree crops, and progress in optimization of coverage patterns to minimize the cost of disruption of *P. citrella*. The applicator (IFM-5051; International Fly Masters) delivered 1 g dollops of SPLAT-CLM into a citrus grove canopy within 2% of the targeted application rate. A field trial conducted in Florida (USA) demonstrated effective disruption (>90%) of male moth catch in traps baited with pheromone lures characterized by high potency following each of four applications of 250 or 500 g ha$^{-1}$ of SPLAT-CLM containing 0.15% (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal. Catch of male moths in pheromone traps deployed as a transect across the border between treated and untreated plots was analyzed to describe the rate of loss of disruption as a function of distance from a treated area. The model was used to estimate a maximum gap of 65 m consisting of untreated rows bounded on both sides by treated rows that could be incorporated into coverage patterns without a significant loss of disruption. A second field trial was conducted to test the feasibility of leaving intentional coverage gaps. No difference in trap catch disruption was observed between plots uniformly treated with SPLAT-CLM and plots where every fifth row (80% coverage) or every fifth and sixth rows (67% coverage) were left untreated. Incorporation of coverage gaps should be effective in reducing product use and overall cost of mating disruption for *P. citrella* in citrus and other species for which mating disruption occurs by a non-competitive mechanism.

## Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a pest of citrus crops throughout the world (Heppner, 1993). Larvae feed within leaves, producing serpentine mines that result in distortion and loss of photosynthetic capacity, thereby reducing yield (Peña et al., 2002). The larvae are a particular concern where citrus bacterial canker, *Xanthomonas axonopodis* pv. citri, occurs. In addition to direct damage, larval feeding increases the susceptibility of leaves to the canker pathogen (Gottwald et al., 2007; Hall et al., 2010). Control of *P. citrella* larvae with insecticides is often ineffective due to the location of larvae within the leaf and the loss of natural enemies (Peña et al., 2002).

The sex pheromone of *P. citrella* was determined to contain three components (Leal et al., 2006; Moreira et al., 2006). Of these (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal (triene) and (*Z*,*Z*)-7,11-hexadecadienal (diene) in a 3:1

---

*Correspondence: Stephen Lapointe, United States Department of Agriculture, Agricultural Research Service, United States Horticultural Research Laboratory, 2001 South Rock Road, Ft. Pierce, FL 34945, USA. E-mail: stephen.lapointe@ars.usda.gov

ratio were shown to be required for attraction to rubber septa in the field (Lapointe et al., 2006, 2009). Control of *P. citrella* by mating disruption using the natural blend has proven effective at very low rates of pheromone deployment (Stelinski et al., 2008). Furthermore, either pheromone compound alone was shown to be capable of disrupting mating in field trials using an emulsified wax formulation (SPLAT-CLM™; ISCA Technologies, Riverside, CA, USA) for slow release of *P. citrella* pheromone applied to citrus trees (Lapointe et al., 2009). The triene was ca. 13 times more effective than the diene alone, and was as effective as, or more effective than, the natural 3:1 blend (Lapointe et al., 2009, 2011). As a result, SPLAT-CLM is now formulated with the triene component only as the active ingredient. However, the cost of synthesis and purification of the triene compound makes SPLAT-CLM an expensive option for *P. citrella* control if applied uniformly to citrus at the recommended rate of 500 g ha$^{-1}$.

SPLAT is a flowable formulation of an emulsified wax compound designed to provide slow release of semiochemicals and allow for mechanical application. Previous studies have shown that droplets of SPLAT provide sustained release of *P. citrella* pheromone (Lapointe et al., 2009; Stelinski et al., 2010). For successful adoption of SPLAT-CLM in commercial citrus, two obstacles must be overcome. An efficient method of applying SPLAT to large areas is needed to minimize application costs and to deliver SPLAT in a manner that maintains the three-dimensional shape of the droplet to avoid overly rapid loss of pheromone. The second obstacle is related to the cost of the pheromone. Synthesis of (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal largely determines the cost of SPLAT-CLM. While improvements in the synthetic protocol and scaling of production can be expected to contain cost to an extent, maximizing longevity and optimizing coverage will probably contribute more to improving the cost effectiveness of *P. citrella* mating disruption. Our research and development efforts are aimed at overcoming these obstacles. Our objective here was two-fold: to test a novel delivery device that allows for precise delivery of uniform droplets of SPLAT, and to determine the effect of SPLAT-CLM on trap catch disruption for control of *P. citrella* in commercial citrus groves. An ancillary goal was to describe the decay of trap catch disruption (attenuation) that occurs with increasing distance from grove areas treated with SPLAT-CLM. Our interest in border effects was to identify application methods that could reduce the cost of mating disruption with SPLAT-CLM by leaving untreated swaths as has been suggested for mating disruption of the gypsy moth, *Lymantria dispar* (L.) (Tcheslavskaia et al., 2005).

## Materials and methods

### SPLAT applicator

SPLAT containing 0.15% (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal (SPLAT-CLM™; ISCA Technologies) was applied using an applicator designed and fabricated in collaboration with International Fly Masters (Fort Pierce, FL, USA). The applicator (IFM 5051) consisted of a pair of computer-controlled variable speed peristaltic pumps that drew the SPLAT compound from reservoirs suspended above the pumps and delivered it to nozzles located above blowers on either side of a frame mounted to a tractor by a three-point hitch. The SPLAT droplets fell by their own weight into the airflow from the blowers and were lofted into the tree canopy. The weight of the SPLAT droplets was calibrated to 1 g by varying the size of the nozzle opening. The weight and number of droplets per unit area were based on results previously reported (Lapointe et al., 2009, 2011). The entire application apparatus was raised or lowered as needed. The angle, as well as the speed, of the blowers could also be varied to control the area of the canopy targeted to receive the SPLAT dollops. Pump speed was controlled using a Raven Pro field computer (Raven Industries, Sioux Falls, SD, USA) that integrated GPS telemetry and provided compensation for vehicle speed.

Applications were made on 5 April, 3 June, 2 August, and 8 October, 2010. SPLAT-CLM was under development during 2010 and therefore the characteristics of the product varied over the year in response to our field experience. The first application was considered too thin [160 000 centipoise (cP)], and subsequent applications were made with a higher viscosity product (300 000 cP). Viscosity measurements were made using a Brookfield-LV-DV-11 programmable viscometer and an LV-4 spindle (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) running at 1 r.p.m., 24.5–25.8 °C.

### Longevity and attenuation trial

Field tests of experimental formulations of SPLAT-CLM were conducted at Emerald Grove (27°28′N, 80°38′W), courtesy of The Packers of Indian River, located in northwestern St. Lucie County, FL, USA during 2010. Three blocks (ca. 183 × 1 550 m or 28 ha each) of actively managed, mature groves of Flame grapefruit on Swingle rootstock were divided into treatment plots, controls, and buffer areas between treatments. Information on pesticide spray applications during the study was obtained from the grower. Thirteen applications of abamectin were made at the Emerald Grove to control *P. citrella* during the period from 5 May through 20 September. Insecticides targeting other arthropod pests applied during the experiments reported here included aldicarb, chlorpyrifos, fenbuconazole, imidacloprid, petroleum oil, pyriproxifen, spirodiclofen, and thiamethoxam. Trees were hedged and topped at between 4 and 5 m in double-row beds spaced 3.8 m between trees within rows and 7.6 m between rows. The blocks were divided into nearly square plots (183 × 191 m) consisting of 13 rows of 48 trees per row. SPLAT-CLM containing 0.15% (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal was applied at two rates (250 and 500 g ha$^{-1}$) with four replicates. Treatment plots were randomly assigned and intercalated with untreated plots of equal dimensions. To assess disruption of the males' ability to orient to an attractive pheromone blend, traps (Pherocon VI; Trécé, Adair, OK, USA) baited with rubber septa loaded with the 3:1 triene:diene blend (Citralure™; ISCA Technologies) were deployed in the center rows of treated and untreated plots. These lures are highly potent with respect to attracting male *P. citrella* for periods over 6 weeks (Lapointe & Leal, 2007). Three traps were deployed in the center rows of each SPLAT-CLM-treated plot and on the center tree of each of 13 untreated plots. Trap liners were replaced approximately weekly and lures were replaced every 6 weeks. Trap catch was calculated as the number of male *P. citrella* per trap per day.

To study the dynamics of trap catch disruption in the vicinity of the borders of plots treated with SPLAT-CLM, transects consisting of six traps were deployed across the border of each treated plot. The distance between transect traps was 25 m, to minimize interactions between traps. Sticky trap liners were replaced weekly. Trap catch disruption was expressed as the number of male *P. citrella* caught in traps divided by the trap catch in untreated (control) plots. To assess the decay of trap catch disruption with increasing distance from the border of treated areas, trap catch data were averaged over 20 dates corresponding to those sampling dates when trap catch disruption exceeded 50% (22 April–4 May, 11 June–16 July, 11 August–23 September, and 15 October–5 November). These values were plotted against distance from the border of the treated area and linear regression was used to describe the decay function.

### Intentional gaps trial

A trial was conducted in October, 2010 at TRB Groves (27°01′N, 81°46′W) in northern Charlotte County, FL, USA to explore the potential for inclusion of intentional coverage gaps to reduce the cost of SPLAT-CLM applications to growers. A low-viscosity SPLAT-CLM formulation was applied using two coverage patterns (67 and 80%) at two nominal application rates of 0.5 and 1.0 kg SPLAT-CLM ha$^{-1}$. As used here, the nominal rate refers to the rate applied to treated areas while the effective rate is the rate averaged over treated and untreated areas within a given treatment. To achieve the coverage patterns, we applied SPLAT-CLM to four rows of Duncan grapefruit (7.3 × 3.7 m spacing between and within rows, respectively) at the nominal rates and skipped one row in a repeated pattern to achieve 80% coverage (effective rates of 400 and 800 g ha$^{-1}$), and we treated four rows at the nominal rates and skipped two rows for 67% coverage (effective rates of 335 and 667 g ha$^{-1}$). Each plot (replication) consisted of a total of 24 rows (205 × 176 m) for the 80% coverage (4-1-4-1-4-1-4-1-4 where four rows were treated and one skipped) and a total of 28 rows (205 × 205 m) for the 67% coverage (4-2-4-2-4-2-4-2-4 where four rows were treated and two skipped). The area of individual plots was ca. 2 ha. Additional plots consisted of 24 rows without skips treated at 0.5 or 1.0 kg SPLAT-CLM ha$^{-1}$, and untreated plots consisted of 24 untreated rows. Each row in all plots contained 32 trees. Plots were replicated three times. Four buffer rows were left untreated between all plots. To assess trap catch disruption, Pherocon VI (Trécé) traps baited with a Citralure septum were placed at the center of untreated plots and treated plots without skips. Baited traps were placed in treated and untreated rows within the plots that contained skipped rows. Sticky trap liners were replaced and counted weekly for a period of 4 weeks following application of SPLAT-CLM. Mean trap catch disruption for each trap collection date was calculated and compared by analysis of variance (ANOVA) with nominal rate (0.5 or 1.0 kg SPLAT-CLM ha$^{-1}$) and percent coverage (67, 80, or 100%) as sources of variance.

### Statistical analysis

Treatments (250 or 500 g SPLAT-CLM ha$^{-1}$) were assigned to alternating plots. The slopes of the linear regression of mean trap catch disruption over time up to 6 weeks following each of the four applications were calculated to compare the rates of loss of the pheromone component from the SPLAT. Data for percent trap catch disruption were arcsin transformed and slopes were compared.

To generate a regression of trap catch disruption based on distance from the treated plots, data were combined for 20 dates when trap catch disruption exceeded 50% for both the 250 and 500 g ha$^{-1}$ treatments. The two equations were similar and the data were combined to generate a quadratic expression describing the decay of trap catch disruption as a function of distance from the treated plot, expressed as a proportion of the difference between the trap catch of traps located at the center of untreated plots and the mean catch of traps located within treated plots. This relationship was used to generate a model for the decline in trap catch that occurs across an intentional gap with treated areas on both sides assuming an equal and proportional effect from the pheromone-treated areas on either side. To generate a mathematical description of the expected trap catch disruption across an intentional coverage gap, we summed the equation of the quadratic regression that described our data and its inverse expression to generate a parabola that describes the theoretical loss of trap catch disruption that would occur across an untreated gap. The number of *P. citrella* males per trap per day for each sampling date was analyzed by ANOVA; sources of error were nominal rate of application (494 or 988 g ha$^{-1}$), coverage (67, 80, or 100%), and treatment (skipped or treated) nested within coverage.

## Results

### SPLAT Applicator

The IFM 5051 applicator consistently delivered SPLAT within 2% of the targeted rate (250 or 500 g ha$^{-1}$) as determined by the on-board computer and actual weight of the SPLAT applied. The applicator was capable of pumping the higher viscosity SPLAT formulation to as much as 150 ha day$^{-1}$ and generated detailed coverage maps.

### Longevity and attenuation trial

Despite insecticide applications, the *P. citrella* population was high as judged by damage and trap catches, and similar to the flight phenology observed in previous studies (Lapointe & Leal, 2007). Trap catch of male *P. citrella* in untreated areas increased from low levels in April to a high in early August followed by flights in late September through early November (Figure 1). In general, and in spite of intensive insecticide applications at this location that mainly targeted the Asian citrus psyllid (*Diaphorina citri* Kuwayama), the phenology of male leafminer flights was similar to that observed during 2006 at a location with minimal chemical control (Lapointe & Leal, 2007). In this study, a maximum trap catch of 81 males per trap per day occurred during the week of 2 August compared with a maximum trap catch of ca. 200 males per trap per day reported by Lapointe & Leal (2007) during August, 2006.

Trap catch disruption exceeded 95% during the 1st week following each of the four application dates when SPLAT-CLM was applied at 500 g ha$^{-1}$. The initial trap catch disruption in plots treated at 250 g ha$^{-1}$ was more variable, ranging from 75% after the first application of low-viscosity SPLAT, to 95% after the third application on 11 August (Figure 2). Overall, the mean (± SEM; n = 20)

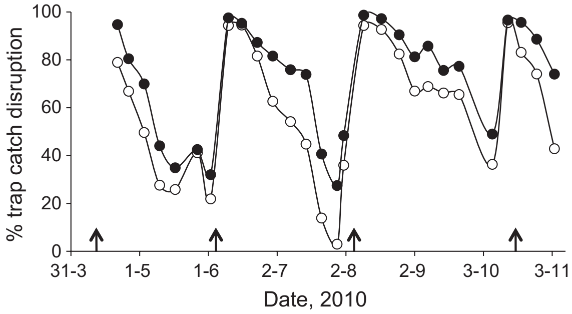

**Figure 2** Disruption of catch of male *Phyllocnistis citrella* in pheromone-baited traps located in plots of mature citrus trees treated with SPLAT-CLM™ at two rates (solid circles: 500 g ha$^{-1}$, open circles: 250 g ha$^{-1}$). Weekly mean trap catch disruption is expressed as one minus the proportion of male moths caught in untreated (control) plots. Arrows indicate dates of application of SPLAT-CLM.

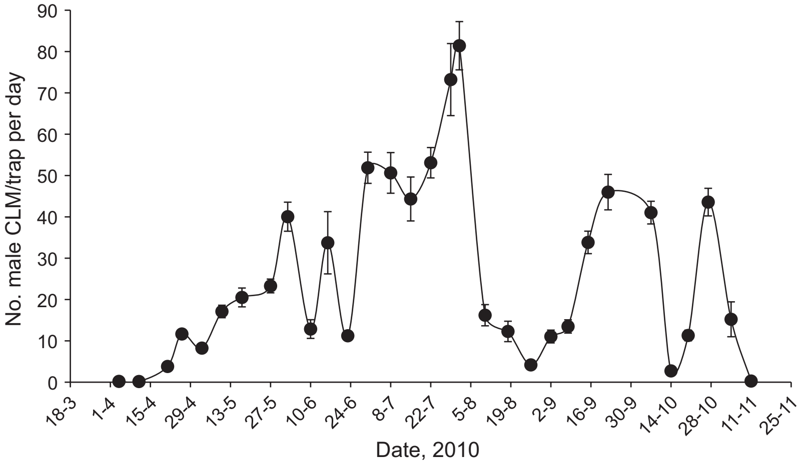

**Figure 1** Mean (± SEM; n = 13) number of male *Phyllocnistis citrella* caught in pheromone-baited traps in untreated plots in a citrus grove during 2010, St. Lucie county, FL, USA.

trap catch disruption at the lower application rate of 250 g $ha^{-1}$ was 15 ± 2% lower compared with trap catch disruption at 500 g $ha^{-1}$. The decline in trap catch disruption over 6 weeks at both application rates was most rapid after the first application with the low-viscosity formulation compared with the subsequent applications of a higher viscosity formulation containing the same amount of pheromone (0.15% a.i.). The slope of the regression of trap catch disruption for the low-viscosity formulation was significantly greater ($F_{2,13} = 109.7$, $P<0.0001$) compared with that for the higher viscosity formulation applied in June and August (Figure 3).

In the transect study, there was no significant difference (ANOVA, $\alpha = 0.05$) between mean trap catch in the central row of the treated plots and the three traps of the transect located within the treated area. We combined these data to estimate trap catch disruption within the treated plots. Transect data generated a model that predicts the width of a gap in coverage that could be intercalated in treated plots while maintaining disruption over the combined area of treated and untreated rows. The decline in trap catch disruption with increasing distance from the plots treated with pheromone was best described by a quadratic curve. Trap catch disruption was inversely proportional to the square of the distance from the signal source (Figure 5).

The transect data describe a situation where pheromone was applied to only one side of a gap in coverage (Figure 4). In an actual application employing intentional coverage gaps, there would be an equivalent but inverse influence of pheromone from the opposite side of the untreated gap (Figure 5). Therefore, we inverted the curve and summed the regression equations to describe a parabola (Figure 6) that describes the loss of trap catch disruption that should occur over a gap of varying size assuming an equal influence from treated areas on both sides of the untreated gap rows. The equation

$$y = -1E - 05x^2 + 0.003x - 0.16$$

describes the parabola used to estimate the maximal gap width that could occur without loss of trap catch disruption by solving for $\Delta_{max} = 0$ (Figure 6). The model suggests that coverage gaps of up to 65 m wide (approximately seven rows, assuming 8 m spacing between rows) could be intercalated between treated areas 200 m wide without significant loss of mating disruption. This would represent an approximate savings of 25% of the pheromone product, applicator time, fuel, etc.

### Intentional gaps trial

We observed a rapid loss of trap catch disruption similar to the disruption obtained after application of the low-viscosity formulation in the longevity trial. Trap catch disruption in plots treated at 500 g $ha^{-1}$ declined to ca. 50% by 4 weeks after application. We saw no significant difference (ANOVA, $\alpha = 0.05$) in disruption between the effective application rates for either nominal rate as coverage was decreased from 100 to 67% (Figure 7) over 4 weeks following application (Table 1).

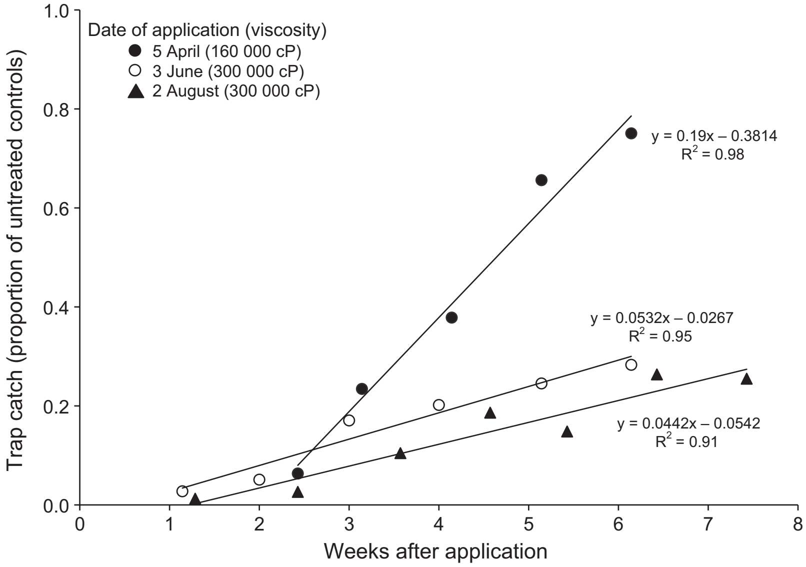

**Figure 3** Slopes of regression lines of trap catch of male *Phyllocnistis citrella* expressed as proportion of the trap catch in untreated plots for ≥6 weeks after three applications of two formulations of SPLAT-CLM of different viscosity.

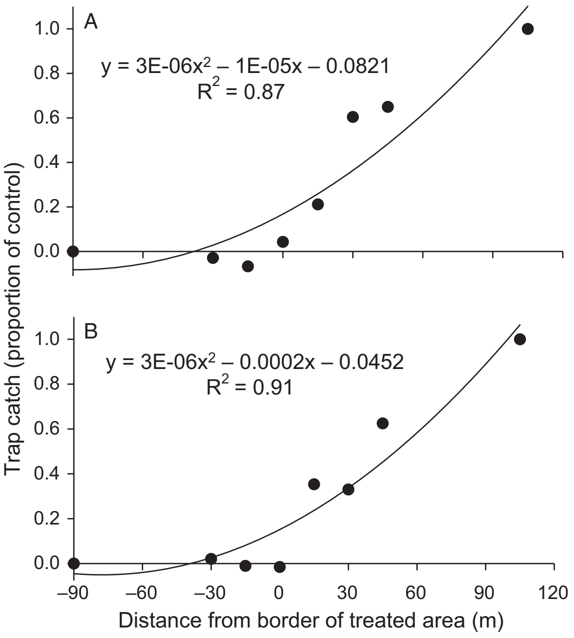

**Figure 4** Mean trap catch expressed as a proportion of controls and regressed on the distance separating traps located at the center of the treated plots (90 m from the edge of treated plots) and traps located at the center of untreated plots (120 m from edge of treated plots): (A) 250 g ha$^{-1}$ and (B) 500 g ha$^{-1}$ SPLAT-CLM.

There was no effect of nominal application rate on trap catch disruption expressed as a percent of that observed in the untreated plots on 8 October ($F_{1,84}$ = 3.015, P = 0.09), 7 days after SPLAT-CLM application. There was no effect of coverage (67, 80, or 100%) ($F_{2,84}$ = 0.090, P = 0.91), nor was there a difference between catch in traps located in treated or skipped rows nested within coverage ($F_{2,84}$ = 1.011, P = 0.37). Similar results were obtained for trap catch numbers collected on 14 October, 13 days after SPLAT-CLM application: there was no effect of nominal rate ($F_{1,84}$ = 1.778, P = 0.19), coverage ($F_{2,84}$ = 0.090, P = 0.91), or treatment (treat/skip) nested within coverage ($F_{2,84}$ = 1.34, P = 0.27) (Table 1).

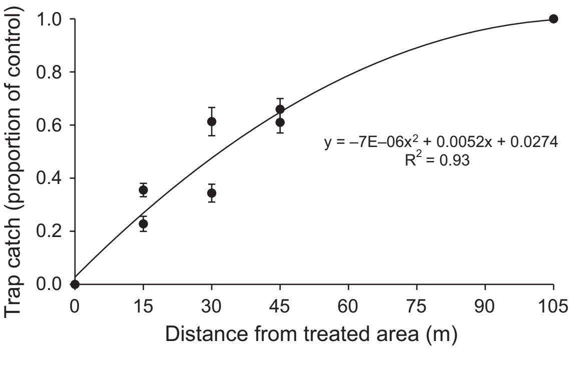

**Figure 5** Attenuation of trap catch disruption with increasing distance from treated area. Trap catch data are expressed as a proportion of the trap catch obtained at the center of untreated plots, 105 m from the edge of treated plots. Data from two experiments (250 and 500 g SPLAT-CLM ha$^{-1}$) were combined; error bars are SEM (n = 18).

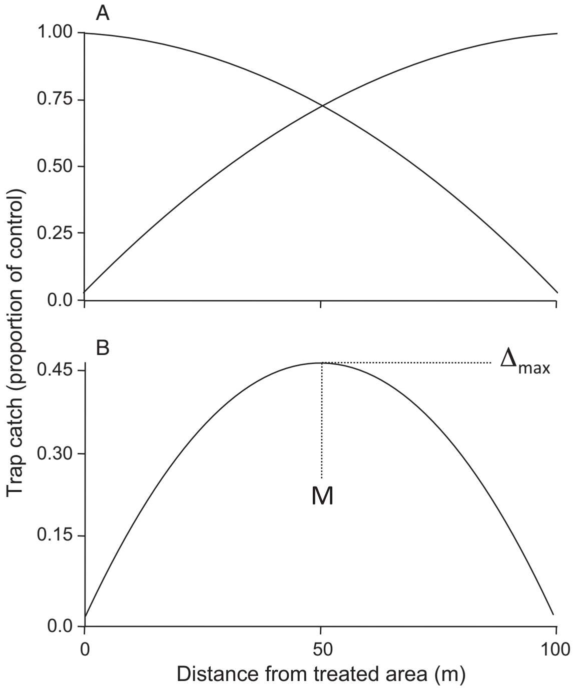

**Figure 6** (A) The regression equation from the data presented in Figure 5 plotted with the inverted relation to simulate the influence of pheromone treatments on either side of an untreated coverage gap. (B) The quadratic equations in (A) were summed to produce a parabola representing a model of expected trap catch attenuation across an untreated gap between two areas treated with pheromone (M = midpoint, $\Delta_{max}$ = maximum loss of disruption).

There was a significant effect of nominal rate for trap catch collected on 22 October, 21 days after SPLAT-CLM application ($F_{1,84}$ = 15.725, P = 0.0002). The mean (± SEM) trap catch of plots treated at the nominal rate of 200 g ha$^{-1}$ was 148 ± 13 compared with 55 ± 13 males per trap per day for plots treated at 400 g ha$^{-1}$, corresponding to 18 and 7% of the trap catch from traps located in untreated plots, respectively. There was no effect of coverage ($F_{2,84}$ = 0.647, P = 0.53) or treatment (skip/treat) nested within coverage ($F_{2,84}$ = 0.913, P = 0.41) (Table 1).

The results for 29 October, 28 days after SPLAT-CLM application, were similar to those for 22 October. There

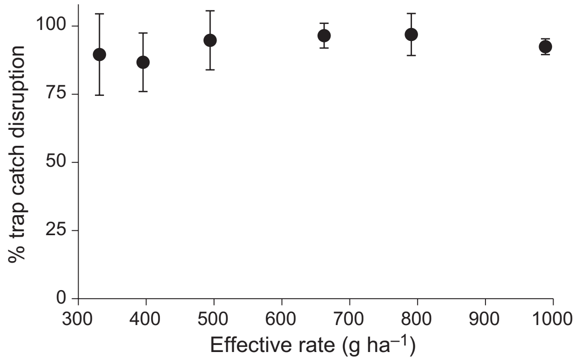

**Figure 7** Mean (± SEM) trap catch disruption over 3 weeks after application of SPLAT-CLM™ to control *Phyllocnistis citrella*. Effective rate is total amount of SPLAT-CLM per unit area and was obtained by varying the number of treated and untreated rows within plots. Effective rates of 494 and 988 g $ha^{-1}$ represent ca. 2-ha plots with uniform application to every row; rates of 331 and 662 g $ha^{-1}$ were achieved by treating 67% of rows at 494 and 988 g $ha^{-1}$ nominal rate (calibrated rate of the applicator), respectively; rates of 395 and 791 g $ha^{-1}$ were achieved by treating 80% of rows at 494 and 988 g $ha^{-1}$ nominal rate, respectively. There was no significant difference among means (ANOVA, $\alpha = 0.05$).

was a significant effect of the nominal rate ($F_{1,84} = 27.247$, $P<0.0001$). The mean (± SEM) trap catch of plots treated at the nominal rate of 200 g $ha^{-1}$ was 713 ± 52 compared with 348 ± 52 males per trap per day for plots treated at 400 g $ha^{-1}$. There was no effect of coverage ($F_{2,84} = 1.814$, $P = 0.17$) nor treatment (skip/treat) nested within coverage ($F_{2,84} = 2.645$, $P = 0.08$). If all non-significant effects (coverage and treatment) were removed from the model leaving only nominal rate, then the nominal rate of application was highly significant across all sampling dates ($P<0.0001$).

## Discussion

The tractor-mounted applicator (IFM-5051) performed well and accurately delivered the desired amount of SPLAT per area of crop. This applicator realizes one of the original goals for SPLAT, i.e., to provide a flowable formulation for mechanical delivery of semiochemicals. Although mechanical applicators for SPLAT have been developed (Stelinski et al., 2007; Jenkins & Isaacs, 2008), previous designs were less precise and efficient in delivery of active ingredient per ha and were of lower capacity. The combination of speed-compensated peristaltic pumps and variable speed fans generated low but sufficient droplet velocity to reach the canopy while minimizing droplet deformation upon impact. The device worked best when used with the higher viscosity SPLAT to avoid spreading of the SPLAT droplet upon contact with leaves or branches. Droplets of the low-viscosity formulation spread upon impact, depositing a thin layer of SPLAT with a higher surface-area-to-volume ratio (SA:V) thereby contributing to more rapid loss of pheromone compared with droplets with a lower SA:V ratio (Atterholt et al., 1998). Applications of higher viscosity SPLAT-CLM produced droplets with a lower SA:V and greater

**Table 1** Mean (± SEM) catch in intentional gaps field trial of *Phyllocnistis citrella* males in traps baited with a natural blend lure located in 2-ha plots with varying coverage patterns at two nominal application rates (500 and 1 000 g $ha^{-1}$) of SPLAT-CLM™

| Rate[1] | Coverage | | | Days after application | | | |
|---|---|---|---|---|---|---|---|
| (g $ha^{-1}$) | (%) | S/T | n | 7 | 13 | 21 | 28 |
| 0 | 0 | S | 6 | 227 ± 32 | 364 ± 44 | 835 ± 177 | 1291 ± 86 |
| 335 | 67 | T | 9 | 12 ± 2 (95)[2] | 38 ± 40 (90) | 154 ± 106 (82) | 967 ± 141 (25) |
| 335 | 67 | S | 12 | 6 ± 1 (98) | 27 ± 10 (93) | 130 ± 82 (84) | 741 ± 96 (43) |
| 400 | 80 | S | 12 | 10 ± 3 (96) | 31 ± 7 (91) | 139 ± 19 (83) | 520 ± 76 (60) |
| 400 | 80 | T | 9 | 12 ± 3 (95) | 51 ± 8 (86) | 191 ± 26 (77) | 742 ± 82 (43) |
| 500 | 100 | T | 3 | 1 ± 1 (99) | 13 ± 5 (96) | 96 ± 26 (88) | 614 ± 87 (52) |
| 670 | 67 | S | 12 | 5 ± 1 (98) | 19 ± 4 (95) | 65 ± 14 (92) | 390 ± 45 (70) |
| 670 | 67 | T | 9 | 3 ± 1 (99) | 9 ± 7 (98) | 37 ± 33 (96) | 220 ± 70 (83) |
| 800 | 80 | S | 12 | 2 ± 1 (99) | 10 ± 4 (97) | 55 ± 14 (93) | 344 ± 38 (73) |
| 800 | 80 | T | 9 | 2 ± 1 (99) | 8 ± 2 (98) | 50 ± 10 (94) | 456 ± 67 (65) |
| 1000 | 100 | T | 3 | 5 ± 4 (98) | 39 ± 8 (89) | 79 ± 16 (90) | 212 ± 34 (84) |

[1] The effective rate of application was varied by including 0, 1, or 2 untreated rows (skips) in treatment plots receiving the nominal rate resulting in an effective rate of 335, 400, 500, 670, 800, or 1 000 g $ha^{-1}$.

[2] Numbers in parentheses indicate trap catch disruption expressed as percentage of the catch in untreated plots; traps were placed either in untreated rows (S) or treated rows (T) with rate/coverage treatment plots.

longevity of trap catch disruption compared with the low-viscosity application (Figure 3). Viscosity will be an important component of quality control to assure maximum longevity of SPLAT-CLM for application by mechanical means.

A 3:1 blend of (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal:(*Z*,*Z*)-7,11-hexadecadienal is required to elicit attraction of male *P. citrella* (Lapointe et al., 2006, 2009). Either component alone caused disruption of male plume following, but 13 times more diene than triene were required to cause the same effect (Lapointe et al., 2009). The triene alone was equally or more effective in disrupting male plume following than the 3:1 blend for periods of ca. 4 weeks (Lapointe et al., 2009). Previously, we reported up to 12 weeks of 90% trap catch disruption with a SPLAT formulation containing a 3:1 blend of treine:diene at a 0.2% loading rate of active ingredient by weight (Stelinski et al., 2010). The previously determined longevities of both the 3:1 blend of triene:diene and diene alone were quantified by applying dollops of SPLAT by hand to citrus foliage resulting in consistent and relatively symmetrical droplets so as to maximize SA:V and longevity of pheromone release (Stelinski et al., 2010). Although the viscosity of SPLAT was not measured in that study, the hand-applied formulation was thicker than the low-viscosity formulation evaluated by mechanical application here.

The longevity observed with the higher viscosity triene formulation in the current study was similar to that quantified previously with SPLAT applied by hand with the diene alone (Stelinski et al., 2010). Although the triene alone disrupted male *P. citrella* effectively by a non-competitive mechanism for up to 4 weeks (Lapointe et al., 2009), it is also possible that the longevity observed with the 3:1 blend of triene:diene is longer because activity of this attractive blend may be characterized by two unique and sequential mechanisms of disruption. Initially and at a high release rate, the 3:1 blend may disrupt male *P. citrella* by a non-competitive mechanism such as camouflage or sensory imbalance followed by competitive attraction at a lower pheromone release rate. A partial, unattractive blend formulation, such as triene alone, would not cause false-plume following at a low release rate, and thus the benefit of competitive attraction below some threshold release rate, when non-competitive disruption is no longer effective, would not be observed. It may be necessary to reconsider use of the natural blend by comparing longevity with the triene only product for maximum longevity of disruption.

The model presented here provides an estimate of what may be possible to achieve in terms of incorporating coverage gaps to reduce the amount of pheromone required to provide effective disruption. It is important to note that our field trial measured the effect of gaps on trap catch disruption for a limited period (4 weeks) after application of SPLAT-CLM. These results need to be validated with the higher viscosity product over a longer period of time. How trap catch disruption persists in application patterns that incorporate intentional gaps over the expected 6–12 weeks of effective disruption that we expect to achieve with a high viscosity SPLAT-CLM will be important in determining the utility of an application strategy that includes untreated rows. However, our data strongly suggest that incorporation of coverage gaps will be effective in reducing product use and overall cost of mating disruption for *P. citrella* in citrus. It is possible that coverage gaps could be effectively incorporated into mating disruption programs for other pest of fruit crops for which non-competitive disruption occurs at economically feasible release rates of pheromone, such as Oriental fruit moth, *Grapholita molesta* (Busck) (Miller et al., 2006).

## Acknowledgements

PeiLing Li, Larry Markle, Kelsey Stevens (USDA-ARS, Ft. Pierce, FL, USA), Wendy Meyer, Siddharth Tiwari, and Ian Jackson (Univeristy of Florida, Lake Alfred, FL) provided technical assistance in the field. Dave Robinson (International Fly Masters) provided prototype machinery and made the field applications. Partial funding was provided by the Specialty Crops Block Grant, USDA, and the Florida Department of Agriculture and Consumer Services. We are particularly grateful to Tom Stopyra (The Packers of Indian River, Ft. Pierce, FL) and David Kemeny (TRB Groves, Punta Gorda, FL) for use of their groves. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

## References

Atterholt CA, Delwiche MJ, Rice RE & Krochta JM (1998) Study of biopolymers and paraffin as potential controlled-release carriers for insect pheromones. Journal of Agricultural and Food Chemistry 46: 4429–4434.

Gottwald TR, Bassanezi RB, Amorim L & Bergamin-Filho A (2007) Spatial pattern analysis of citrus canker-infected plantings in São Paulo, Brazil, and augmentation of infection elicited by the Asian leafminer. Phytopathology 97: 674–683.

Hall DG, Gottwald TR & Bock CH (2010) Exacerbation of citrus canker by citrus leafminer *Phyllocnistis citrella* in Florida. Florida Entomologist 93: 558–566.

Heppner JB (1993) Citrus leafminer, *Phyllocnistis citrella*, in Florida. Tropical Lepidoptera 4: 49–64.

Jenkins PE & Isaacs R (2008) Mating disruption of *Paralobesia viteana* (Lepidoptera: Tortricidae) in vineyards using pheromone deployed in SPLAT-GBM™ wax droplets. Journal of Chemical Ecology 34: 1089–1095.

Lapointe SL & Leal WS (2007) Describing seasonal phenology of the leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) with pheromone lures: controlling for lure degradation. Florida Entomologist 90: 710–714.

Lapointe SL, Hall DG, Murata Y, Parra-Pedrazzoli AL, Bento JMS et al. (2006) Field evaluation of a synthetic female sex pheromone for the leafmining moth *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Florida citrus. Florida Entomologist 89: 274–276.

Lapointe SL, Stelinksi LL, Evens TJ, Niedz RP, Hall DG & Mafra-Neto A (2009) Sensory imbalance as mechanism of mating disruption in the leafminer *Phyllocnistis citrella*: elucidation by multivariate geometric designs and response surface models. Journal of Chemical Ecology 35: 896–903.

Lapointe SL, Stelinksi LL & Robinson RD (2011) A novel pheromone dispenser for mating disruption of the leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Journal of Economic Entomology 104: 540–547.

Leal WS, Parra-Pedrazzoli AL, Cossé AA, Murata Y, Bento JMS & Vilela EF (2006) Identification, synthesis, and field evaluation of the sex pheromone from the citrus leafminer, *Phyllocnistis citrella*. Journal of Chemical Ecology 32: 155–168.

Miller JR, Gut LJ, de Lame FM & Stelinski LL (2006) Differentiation of competitive vs. non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone (Part 2): case studies. Journal of Chemical Ecology 32: 2115–2143.

Moreira JA, McElfresh S & Millar JG (2006) Identification, synthesis, and field testing of the sex pheromone of the citrus leafminer, *Phyllocnistis citrella*. Journal of Chemical Ecology 32: 169–194.

Peña JE, Sharp JL & Wysoki M (2002) Tropical Fruit Pests and Pollinators: Biology, Economic Importance, Natural Enemies, and Control. CABI Press, Wallingford, UK.

Stelinski LL, Miller JR, Ledebuhr R, Siegert P & Gut LJ (2007) Season-long mating disruption of *Grapholita molesta* (Lepidoptera: Tortricidae) by one machine application of pheromone in wax drops (SPLAT-OFM). Journal of Pest Science 80: 109–117.

Stelinski LL, Miller JR & Rogers ME (2008) Mating disruption of citrus leafminer mediated by a non-competitive mechanism at a remarkably low pheromone release rate. Journal of Chemical Ecology 34: 1107–1113.

Stelinski LL, Lapointe SL & Meyer WL (2010) Season-long mating disruption of citrus leafminer, *Phyllocnistis citrella* Stainton, with an emulsified wax formulation of pheromone. Journal of Applied Entomology 134: 512–520.

Tcheslavskaia K, Brewster C, Thorpe K, Sharov A, Leonard D & Roberts A (2005) Effects of intentional gaps in spray coverage on the efficacy of gypsy moth mating disruption. Journal of Applied Entomology 129: 475–480.