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Suppression of citrus leafminer, *Phyllocnistis citrella*, with an attract-and-kill formulation

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Abstract

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a worldwide pest of citrus crops and is responsible for proliferation of citrus bacterial canker, *Xanthomonas axonopodis* (Hasse) pv. *citri* (Gamma Proteobacteria: Xanthomonadaceae). We developed and evaluated an attracticide formulation, termed MalEx, for control of *P. citrella*. MalEx is a viscous paste with UV-protective properties that is dispensed as 50-μl droplets using custom-made calibrated pumps. A formulation containing 0.016% *P. citrella* pheromone [3:1 blend of (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal and (*Z*,*Z*)-7,11-hexadecadienal] and 6% permethrin was found to suppress male response to pheromone in the field better than formulations containing 10× less pheromone. Although formulations without permethrin showed some suppression of male activity because of mating disruption, addition of 6% permethrin to the formulation was required for optimal efficacy. When MalEx, containing 0.016% pheromone and 6% permethrin, was applied at 3 000 point sources ha^{-1}, the application height did not influence efficacy of male *P. citrella* suppression within mature 4-m tall citrus trees. Decreasing the rate of MalEx from 3 000 to 1 500 droplets ha^{-1} reduced efficacy as measured by both male *P. citrella* activity and larval infestation. Although 4 500 droplets ha^{-1} did not result in statistically better efficacy than 3 000 droplets ha^{-1}, there was a noticeable trend for higher efficacy as droplet density increased. Continuous treatment of 0.5-ha blocks of citrus with MalEx over the course of 112 days reduced larval infestation of new flush, as compared with those in untreated control plots, by 3.6–7.2× depending on droplet application density. In laboratory behavioral bioassays, the attractiveness of MalEx droplets to male *P. citrella* was drastically reduced after 21 days of field aging. However, our laboratory investigation confirmed that 100% of males contacting MalEx droplets, aged up to 35 days in the field, were killed within 24 h. Direct observation of male *P. citrella* behavior in the field confirmed that attracted males made contact with droplets. Control of *P. citrella* with MalEx should reduce the number of required broad spectrum sprays for leafminer management in both field and citrus nursery settings.

Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a major worldwide pest of citrus production (Heppner, 1993). Larval feeding within serpentine mines damages leaves, which can result in yield loss (Peña et al., 2000). Furthermore, leaf wounds caused by *P. citrella* larval feeding predisposes trees to infection by citrus bacterial canker, *Xanthomonas axonopodis* (Hasse) pv. *citri* (Gamma Proteobacteria: Xanthomonadaceae), responsible for blemished fruit, premature fruit drop, and tree decline (Graham et al., 2004). Feeding *P. citrella* larvae tear the leaf cuticle, exposing mesophyll to direct infection. Wounds caused by *P. citrella* do not heal readily, which increases the exposure period to the bacterium and its spread by larvae moving throughout feeding galleries (Graham et al., 2004). Feeding larvae within the leaf mines are protected from foliar applications of toxicants rendering insecticidal control of the larval stage difficult or in

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some instances ineffective. When spray programs can be implemented, they may require bi-weekly applications given continual growth of new leaf flush, which is highly detrimental to natural enemy populations of *P. citrella* and other citrus pests (Peña et al., 2002). Development of effective control strategies for *P. citrella* is of critical importance for the leading citrus producing countries, including Brazil and the USA, where citrus canker limits production (Leite & Mohan, 1990).

Recently, the sex pheromone of *P. citrella* was identified (Leal et al., 2006; Moreira et al., 2006). A 3:1 blend of (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal (*Z7Z*11*E*13-16Ald) and (*Z*,*Z*)-7,11-hexadecadienal (*Z7Z*11-16Ald) is highly attractive to males. Effective monitoring protocols using this pheromone have been developed (Lapointe et al., 2006; Stelinski & Rogers, 2008) and the potential for mating disruption of *P. citrella* has been investigated (Mafi et al., 2005; Stelinski et al., 2008).

Pheromone-based mating disruption is highly effective for *P. citrella* control (Stelinski et al., 2008). However, given the high cost of synthesis of the *P. citrella* pheromone components, it is unclear whether mating disruption will be a viable commercial option for management of this pest. Given the species-specific mode of action of mating disruption, high input costs targeting *P. citrella* management alone in worldwide citrus production may be impossible given the emergence of the Asian citrus psyllid, *Diaphorina citri* Kuwayama, as another worldwide pest limiting citrus production (Halbert & Manjunath, 2004). Thus, behavioral modification tools that are both effective and economical are needed for management of *P. citrella*. One potential alternative to mating disruption is the development of an attract-and-kill method for *P. citrella*. There is a large precedent for effective attracticides targeting lepidopteran pests, which combine the use of a very low dose of both synthetic sex pheromone and permethrin insecticide (Charmillot et al., 1996, 2000; Suckling & Brockerhoff, 1999; Krupke et al., 2002; Evenden & McLaughlin, 2004a). Such formulations are typically applied as small droplets, which release pheromone at a rate highly attractive to males. Responsive males follow the plumes from attracticide droplets and can obtain a lethal dose of toxicant upon contact with the source of attractant. Although some investigations have shown that formulations developed with the intent of controlling a lepidopteran pest by attract-and-kill actually function by mating disruption (Evenden & McLaughlin, 2004a), others have proven that the addition of toxicant improved control over formulations containing only pheromone active ingredients (AI) (Charmillot et al., 1996; Suckling & Brockerhoff, 1999). An advantage of attract-and-kill over mating disruption is that killed males are permanently removed from the breeding population. As competitive attraction is population density dependent (Miller et al., 2006), attract-and-kill should prove more effective than mating disruption by false plume following at high-pest densities. Furthermore, attract-and-kill formulations use less pheromone AI per crop area than mating disruption formulations.

In the past decade, a gel matrix with UV-protective properties was developed as an attracticide for lepidopteran pests. This formulation was registered in Switzerland under the trade name Sirene (Charmillot & Hofer, 1997) and subsequently as LastCall in the USA, Europe, and South Africa (Evenden & McLaughlin, 2004a). The original target pest of this formulation was the codling moth, *Cydia pomonella* L. (Charmillot & Hofer, 1997); however, LastCall has been adopted for multiple other pests since then (Brockerhoff & Suckling, 1999; Evenden & McLaughlin, 2004a; Nansen & Phillips, 2004; Maxwell et al., 2006; Evenden et al., 2008). The goal of this investigation was to develop effective attract-and-kill for *P. citrella* by adopting the controlled release gel, termed MalEx, which is the currently licensed name for a formulation similar to LastCall. The specific objectives were to determine the effect of (1) pheromone dosage; (2) presence of toxicant; (3) droplet density per ha of crop; (4) placement height within the tree canopy; and (5) formulation aging on suppression of male *P. citrella* activity, leaf infestation, as well as *P. citrella* mortality in both field and laboratory investigations. In addition, behavioral observations were conducted to document attraction of *P. citrella* males to droplets of MalEx in the field.

Materials and methods

Attracticide formulation

The formulation used in both field and laboratory experiments, termed MalEx (Alpha Scents, Bridgeport, NY, USA), intended for control of *P. citrella* was comprised of an inert viscous proprietary paste (94%), permethrin (6% by weight), and *P. citrella* pheromone (0.016% by weight). The pheromone components of *P. citrella*, *Z7Z*11*E*13-16Ald and *Z7Z*11-16Ald, were synthesized as described previously (Leal et al., 2006; Moreira et al., 2006). *Z7Z*11*E*13-16Ald was 94 and 90% chemically and isomerically pure, respectively, and *Z7Z*11-16Ald 86 and 72%, respectively. A 3:1 blend of these components, which is optimal for attracting males (Leal et al., 2006; Moreira et al., 2006), was loaded into the MalEx formulation. MalEx was dispensed from custom-made pumps calibrated to deposit 50 μl droplets. This base formulation was slightly modified for one experiment described below to determine the effect of pheromone dosage and presence of insecticide on efficacy against *P. citrella*.

Effect of pheromone dosage and insecticide

The objective of this experiment was to investigate the effect of pheromone dosage and presence of permethrin on efficacy of MalEx in suppressing catch of male *P. citrella* in pheromone-baited traps positioned in treated plots. A total of four MalEx treatments were formulated. Two loading dosages of pheromone (0.0016 and 0.016% by weight) were formulated with and without 6% permethrin by weight.

The experiment was arranged as a randomized complete block design. Treatments were randomly assigned to five 0.5-ha replicate plots in an 8-year-old orange orchard [*Citrus sinensis* (L.) var. Valencia (Rutaceae)] in Clermont, FL, USA(28°N, 81°W). Trees were planted on a 3.0 × 6.0 m spacing with a 4.0 m-average canopy height. Replicate plots were separated by 60 m and blocks of treatments were separated by 80 m. MalEx formulations were randomly assigned to replicate plots and dispensed at a rate of 3 000 deposits ha^{-1} (approximately 6 droplets per tree). Droplets were placed onto bark of tree branches 2.0 m above ground level. Treatments were applied on 8–9 June 2008 and the experiment was conducted through 29 June 2008, when efficacy began to noticeably decrease as measured by moth catch in pheromone traps. Control plots were left completely untreated and no additional insecticides were sprayed in this orchard during the course of the experiment. Male *P. citrella* activity in experimental plots was quantified using two pheromone traps (LPD Scenturion Guardpost; Suterra, Bend, OR, USA) deployed within each replicate plot. Traps were placed six trees apart in the central row of each plot. All traps were baited with a single red rubber septum lure loaded with 0.1 mg *Z*7*Z*11*E*13-16Ald and 0.03 mg *Z*7*Z*11-16Ald, and hung at least 1.0 m from the nearest MalEx droplet, at ca. 1.5–2 m above ground level (Stelinski & Rogers, 2008). Moths captured in traps were counted and removed weekly.

Effect of droplet density

The objective of this investigation was to determine the effect of MalEx droplet density on *P. citrella* control efficacy. The formulation used was the base formulation described above containing 6% permethrin and 0.016% pheromone, because this was the most effective treatment in the initial experiment (see Results). The treatments compared were 0, 1 500, 3 000, and 4 500 MalEx droplets ha^{-1}. Treatments were randomly assigned to 0.5-ha replicate plots arranged in a randomized complete block design with five replicates in the citrus orchard described above. Spacing between treatments and blocks was as described above. The experiment was initiated by applying treatments on 6–7 July 2008. Treatments were re-applied 7–8 August. The experiment was terminated on 29 September 2008. Treatments were evaluated weekly using pheromone monitoring traps according to the procedure described above. Pheromone lures were replaced every 7.5 weeks (Lapointe & Leal, 2007). In addition, damage to newly flushed leaves was assessed half way through (10–12 August) and at the end (28–29 September) of the trial. Damage was assessed by inspecting leaf flush samples chosen at random. For each tree, 10 samples were inspected from mid-canopy (2.5 m) and 10 from lower canopy (1.0 m) on 20 trees per replicate block (2 000 flush samples per treatment). The number of shoots per tree containing live mining *P. citrella* larvae was recorded.

Effect of droplet height within the tree canopy

The objective of this experiment was to determine the effect of height of MalEx droplet placement on suppression of *P. citrella* male flight as measured by pheromone trapping. The formulation used was the base formulation described above containing 6% permethrin and 0.016% pheromone. All treatments were applied at a rate of 3 000 MalEx droplets ha^{-1} or approximately 6 droplets per tree. The application heights compared relative to the ground were 0.6, 2.0, and 3.5 m within the citrus orchard described above, which was comprised of trees averaging 4 m in height. Control plots were left untreated. Treatments were randomly assigned to 0.1-ha replicates arranged in a randomized complete block design with five replicates. Replicate plots were separated by 40 m and blocks of treatments were separated by 50 m. Suppression of male *P. citrella* flight activity was measured with weekly assessments of pheromone traps. One pheromone trap was deployed centrally per replicate plot ca. 1.5–2 m above ground level according to the procedures described above. The experiment was conducted 9–30 July 2008.

Effect of droplet aging

The objective of this experiment was to asses the behavioral response of male *P. citrella* to droplets of MalEx following various durations of field aging. Four formulations of MalEx were compared (Table 1) and 40 droplets were evaluated per formulation and aging period combination. Individual 50-µl droplets of MalEx were deposited onto 1 × 1 cm pieces of aluminum foil. Aluminum foil pieces were affixed using thumb tacks to 10 × 100 cm pieces of wood particle board. Particle boards containing MalEx samples were affixed with wire into the canopies of eight citrus trees in the orchard described above. Droplets were deployed on several dates 5–25 August 2008, to establish a staggered aging schedule for each formulation. This allowed testing of each formulation and aging period treatment on a given day until sufficient replicates were accrued for each formulation and day of aging treatment

Table 1 Behaviors of *Phyllocnistis citrellla* in response to various formulations of MalEx following three durations of droplet aging in the field

	% attraction					% contact				
		0.0016% AI[1]	0.016% AI	0.0016% AI	0.016% AI		0.0016% AI	0.016% AI	0.0016% AI	0.016% AI
Days of aging	Control	No permethrin		With permethrin		Control	No permethrin		With permethrin	
0	0.0Ab	89.4Aa	92.7Aa	90.2Aa	94.7Aa	0.0Ab	65.3Aa	72.6Aa	67.9Aa	75.3Aa
7	0.0Ac	74.5Aa	89.4Aa	67.2Bb	90.4Aa	0.0Ac	61.7Aa	68.7Aa	49.5Bb	68.3Aa
21	0.0Ac	67.3Ba	71.4Ba	54.7Bb	77.1Ba	0.0Ac	51.5Ba	56.4Ba	38.4Bb	54.6Ba
35	0.0Ac	19.0Cb	29.8Ca	12.1Cb	14.2Cb	0.0Ac	9.6Cb	16.1Ca	4.8Cb	6.3Cb

Means followed by the same uppercase letter within a column or the same lowercase letter within a row (separately for % attraction and % contact) are not significantly different (G^2 test of homogeneity: $P>0.05$).
[1]*Phyllocnistis citrella* pheromone, 3:1 *Z*7*Z*11*E*13-16Ald and *Z*7*Z*11-16Ald.

combination. Droplets were collected at random for behavioral assays after 7, 21, and 35 days of field aging. In addition, 40 fresh droplets were evaluated to establish the behavioral response of males to MalEx prior to field aging.

Behavioral responses were evaluated in a Y-tube assay according to the procedures described in Wenninger et al. (2008). In brief, the Y-tube consisted of a 14-cm-long stem and two 10-cm-long arms, each with a 2-cm inner diameter. A screened glass plug at the base of the stem was used to introduce insects into the Y-tube. Odor sources were placed at the upwind end of one arm of the Y-tube such that attracted males were able to make contact with MalEx droplets. Charcoal-filtered, humidified air was metered through the two arms of the Y-tube via polytetrafluoroethylene tubing at 500 ml min^{-1}. The air exiting the wire screen plug at the base of the stem was 26–28 °C and 70–85% r.h.; light generated by two 95-W fluorescent bulbs (model F96T12; Philips, Eindhoven, the Netherlands) was measured at ca. 3 200 lux just above the branching point of the Y-tube. The position (left or right) of the test odor source was randomly selected for the first of any set of observations and alternated thereafter. For the blank control treatment, a 1 × 1 cm piece of clean aluminum foil without MalEx was inserted into one of the arms of the Y-tube per replicate moth tested. Between assays, glassware was rinsed thoroughly with acetone and deionized water, soaked in hot soapy water for 30–60 min, and kept in a drying oven (150 °C) for at least 2 h (and usually overnight) before re-use.

Phyllocnistis citrella were collected at the pupal stage from an infested greenhouse (Lake Alfred, FL, USA; 28°N, 81°W) maintained at 26 °C and 60% r.h. Pupae were sorted by sex (Jacas & Garrido, 1996) and males were allowed to emerge in 1-l plastic cages containing 5% (wt/vol) sucrose in plastic cups with cotton dental wicks protruding from their lids. Adult males were maintained for 2–3 days at 26 °C, 60% r.h., and an L12:D12 photocycle prior to testing. All tests were initiated 1.0–1.5 h prior to the end of scotophase to match the time when *P. citrella* are sexually active (Jacas & Peña, 2002). Male moths (n = 40 per treatment) were released individually into the glass plug at the base of the stem of the Y-tube, and their behavior was observed over a 3-min period. The response of one male *P. citrella* was evaluated per droplet. Each MalEx formulation treatment was presented to previously untested male *P. citrella* on each day of testing until 40 replicates were accrued per treatment combination. The first choice of each moth was recorded as the arm in which the male first entered ca. 1 cm into the arm. In addition, the number of males contacting MalEx droplets was recorded. Following each assay, the male was carefully removed from the Y-tube and placed individually into a 240-ml plastic cup containing a moistened dental cotton wick that was sealed with a perforated lid. Each cup was marked to indicate treatment and whether or not males made contact with the droplet of MalEx. Cups were maintained in an environmental chamber set at 26 °C and 60% r.h. for 24 h, after which mortality was assessed. All insects laying on their back and remaining motionless after prodding with a fine probe were scored as dead.

Behavioral observations in the field

Male *P. citrella* behavior was observed in the field to determine whether male moths approached droplets of MalEx. Observations of MalEx droplets in tree canopies were conducted for approximately 2 h each night between 21:00 and 23:00 hours, a period when male *P. citrella* are known to exibit response toward pheromone sources in the field in Florida (Stelinski & Rogers, 2008). Observations were conducted on eight nights between 9 and 29 June. Observations were conducted in the treatment plots described

above designed to investigate the effect of pheromone loading and presence of permethrin. 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. During observations, data were dictated into a hand-held microcassette audio recorder by an investigator standing ca. 0.3 m from the MalEx droplet under observation. Observations after dusk employed night-vision goggles (Model 3250; Rigel, DeWitt, IA, USA) as described by Stelinski et al. (2004).

Statistical analysis

Moth catch data were subjected to analysis of variance (ANOVA) after transformation to $\ln(x + 1)$, which normalized the distributions and homogenized variances. Because flush injury trends were nearly identical at the mid- and end-points of the droplet density study, injury data from the two sampling dates were combined and arcsine transformed prior to ANOVA. Data on the mean number of males approaching various formulations of MalEx in the field were also subjected to ANOVA. A logistic model was used to measure the probability that male *P. citrella* would approach droplets of various formulations of MalEx using Proc GENMOD in SAS (SAS Institute, 2000). Subsequently, analyses of numbers of male moths responding were carried out using the G statistic, testing the null hypothesis of no preference (Sokal & Rohlf, 1981). Proportions of moths responding were compared separately between formulation treatments within each aging period and between the aging periods tested within each formulation treatment. In all cases, the significance level α was 0.05.

Results

Effect of pheromone dosage and insecticide

Fewer male *P. citrella* were captured in plots treated with MalEx containing permethrin, at both pheromone concentrations tested, than in control plots ($F_{4,16} = 11.3$, $P<0.0001$; Figure 1). Catch of male *P. citrella* was statistically equivalent between the two pheromone concentrations tested when MalEx formulations contained permethrin ($P>0.05$; Figure 1). More male *P. citrella* were caught in plots treated with MalEx without permethrin, at both pheromone concentrations tested, than in plots treated with MalEx containing permethrin ($P<0.0001$; Figure 1). Catch of male *P. citrella* was reduced, compared with the control, in plots treated with MalEx not containing permethrin at the 0.016% pheromone concentration ($P<0.0001$), but not at the 0.0016% concentration (Figure 1).

Effect of droplet density

Fewer male *P. citrella* were captured in plots treated with each density of MalEx attracticide tested than in control plots ($F_{3,12} = 12.2$, $P<0.001$; Figure 2A). Male catch was lower in plots treated with 4 500 and 3 000 droplets ha^{-1} than in plots treated with 1 500 droplets ha^{-1} ($P<0.001$; Figure 2A). Although there was no statistical difference in male catch suppression between the two highest density treatments tested ($P>0.05$; Figure 2A), nearly 15× fewer male *P. citrella* were caught in plots treated with 4 500 droplets ha^{-1} than in plots treated with 3 000 droplets ha^{-1}.

Infestation of citrus leaves by larval *P. citrella* was lower in plots treated with 3 000 and 4 500 MalEx droplets ha^{-1} than in control plots ($F_{3,12} = 6.8$, $P<0.025$; Figure 2B). However, there was no statistical difference in *P. citrella* infestation between plots treated with 1 500 MalEx droplets ha^{-1} and control plots ($P>0.05$; Figure 2B). Although there was no statistical difference ($P>0.05$) in leaf infestation between the two highest density treatments tested (Figure 2B), the infestation level observed with 4 500 droplets ha^{-1} was half of that observed with 3 000 droplets ha^{-1}.

Effect of droplet height within the tree canopy

Catch of male *P. citrella* was lower in plots treated with MalEx attracticide, at each canopy height tested, than in control plots ($F_{3,12} = 7.4$, $P<0.01$; Figure 3). When applied at a density of 3 000 droplets ha^{-1}, suppression of male *P. citrella* catch was statistically equivalent among the three droplet application heights compared ($P>0.05$; Figure 3).

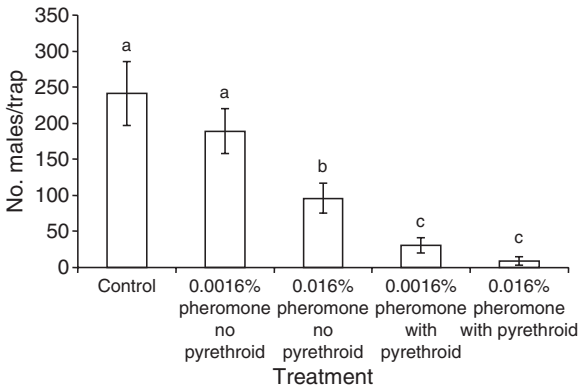

Figure 1 Mean (± SE) captures of male *Phyllocnistis citrella* in pheromone-baited traps as influenced by pheromone concentration as well as presence of permethrin in MalEx attracticide applied as 3 000 droplets ha^{-1}. Bars labeled with the same letter are not significantly different (ANOVA followed by LSD test, $\alpha = 0.05$).

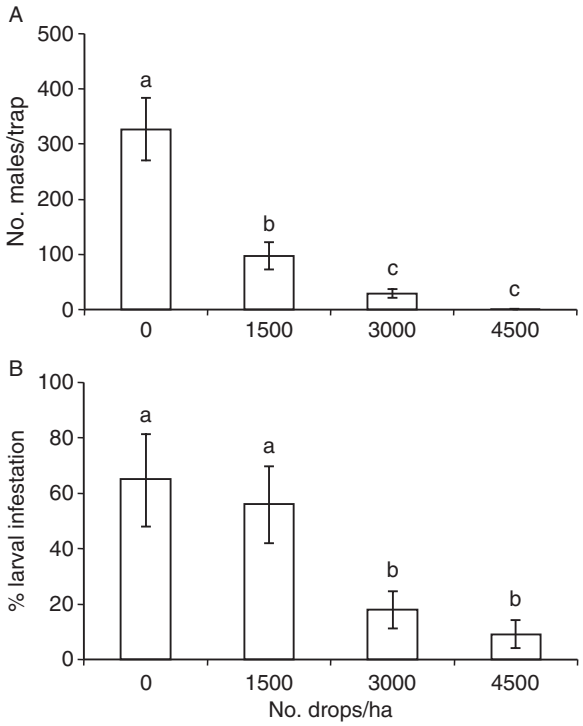

Figure 2 (A) Mean (± SE) captures of male *Phyllocnistis citrella* in pheromone-baited traps and (B) mean percent leaf flush infestation by *P. citrella* larvae as influenced by droplet density of MalEx containing 0.016% pheromone and 6% permethrin. Bars within a panel labeled with the same letter are not significantly different (ANOVA followed by LSD test, $\alpha = 0.05$).

Effect of droplet aging

Following each aging period, more male *P. citrella* were attracted (G = 6.8, d.f. = 1, P<0.01) to and contacted (G = 7.2, d.f. = 1, P<0.01) MalEx droplets, with or without permethrin and regardless of the pheromone concentration tested, than blank controls (Table 1). Approximately 90% of released male *P. citrella* were attracted to fresh droplets of each MalEx formulation and over 65% of those exhibiting attraction also contacted droplets within the 3-min assay period (Table 1). There were no statistical differences between *P. citrella* responses to the various MalEx formulations when males were assayed to fresh droplets (day 0) (G = 0.9, d.f. = 1, P = 0.2; Table 1). At 7 days of field aging, fewer male *P. citrella* were attracted to and contacted MalEx droplets containing 0.0016% pheromone and permethrin than on day 0 (G = 5.9, d.f. = 1, P<0.01); however, male responses to the other formulations tested were statistically equivalent on days 7 and 0 (G = 1.2, d.f. = 1, P = 0.1; Table 1). Behavioral responses of male *P. citrella* to droplets of each MalEx formulation were lower after 21 days of field aging than on day 0 (G = 6.3, d.f. = 1, P<0.01; Table 1). On day 21, fewer male *P. citrella* were attracted to and contacted MalEx droplets containing permethrin and 0.0016% pheromone than to the other formulations tested (G = 4.8, d.f. = 1, P<0.01; Table 1). Male responses to droplets of each MalEx formulation were lower on day 35 than on day 21 (G = 8.4, d.f. = 1, P<0.01; Table 1). After 5 weeks of field aging, <20% of the male *P. citrella* assayed contacted MalEx droplets in the Y-tube olfactometer.

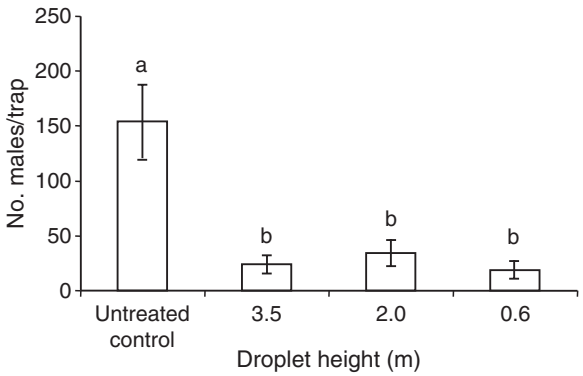

Figure 3 Mean (± SE) captures of male *Phyllocnistis citrella* in pheromone-baited traps as influenced by droplet application height above ground level of MalEx containing 0.016% pheromone, 6% permethrin, and applied as 3 000 droplets ha^{-1}. Bars labeled with the same letter are not significantly different (ANOVA followed by LSD test, $\alpha = 0.05$).

For the two formulations containing permethrin, all male *P. citrella* contacting MalEx droplets died within 24 h, irrespective of the duration of field aging. However, mortalities of male *P. citrella* contacting droplets of the two MalEx formulations without permethrin or the blank control were <3% after 24 h. Also, mortality of male *P. citrella* attracted to but not contacting MalEx droplets with permethrin was <3% after 24 h.

Behavioral observations in the field

Male *P. citrella* were observed orienting to each formulation of MalEx droplets (Table 2). Fewer male *P. citrella* were observed approaching droplets of MalEx loaded with 0.0016% pheromone without permethrin compared with the other formulation treatments ($F_{3,12} = 8.5$, P<0.001; Table 2).

Discussion

Collectively, our results demonstrate that an attracticide formulation combining pheromone and permethrin suppressed flight activity of male *P. citrella* to synthetic point sources of pheromone and reduced larval infestation of leaves. Highly effective mating disruption of male

Table 2 Mean (± SE) numbers of *Phyllocnistis citrella* observed visiting droplets from various formulations of MalEx per night in the field

Treatment	No. males
0.0016% pheromone, no permethrin	12.3 ± 2.5b
0.016% pheromone, no permethrin	27.0 ± 4.7a
0.0016% pheromone, with permethrin	23.1 ± 3.0a
0.016% pheromone, with permethrin	19.5 ± 1.8a

Means followed by the same letter are not significantly different (ANOVA followed by LSD test: $P>0.05$).

P. citrella and associated reduction of leaf infestation has been documented for up to 221 days with two deployments of 1.5 g pheromone AI ha^{-1} (Stelinski et al., 2008). A single deployment of 3 000 droplets of MalEx, containing 0.016% pheromone by weight, amounts to deploying approximately 24 mg pheromone ha^{-1}. To achieve 221 days of comparable efficacy, approximately 10.5 deployments of this MalEx formulation would be required. This would require deploying 0.25 g pheromone AI ha^{-1} over the 31.5-week interval. Thus, the base MalEx formulation developed herein for *P. citrella* control could reduce cost of application as compared with mating disruption by requiring nearly 12× less pheromone ha^{-1} of crop per season. The drawback, however, is that MalEx would require approximately 5× as many deployments than mating disruption dispensers to achieve a comparable duration of efficacy. This discrepancy is likely because of the difference in pheromone loading rate per release source when comparing mating disruption dispensers evaluated for *P. citrella* (1.0 mg loading per dispenser; Stelinski et al., 2008) vs. a single droplet of MalEx (8 μg loading per dispenser). Also, the loading rate of pheromone AI required for effective attract-and-kill of *P. citrella* in the MalEx formulation is approximately 10× lower than that required for other lepidopteran species controlled by similar formulations (Charmillot et al., 1996, 2000; Suckling & Brockerhoff, 1999; Krupke et al., 2002; Evenden & McLaughlin, 2004a).

After 5 weeks of field aging, droplets of the base formulation of MalEx were still attractive to male *P. citrella*. However, by this point they attracted approximately one-fifth of the number of males that were attracted by fresh droplets. Congruently, our field experiments proved that MalEx droplets effectively suppressed male *P. citrella* captures in pheromone traps for ca. 1 month. This duration of attractiveness is lower than that observed with attracticide formulations for other moth species. An attracticide formulation targeting *Epiphyas postvittana* (Walker) remained highly attractive to males over a 3-month period (Brockerhoff & Suckling, 1999), whereas another formulation targeting *Caloptilia fraxinella* (Ely) remained attractive for at least 5 weeks (Evenden et al., 2008). A major factor limiting the utility of MalEx as a practical control tool for management of *P. citrella* will be the duration it remains attractive to males in the field. The current formulation will require monthly reapplications to maintain efficacy. Extending this duration of attractiveness is currently under investigation.

Reducing the pheromone loading rate in MalEx to 0.0016% by weight decreased efficacy of male *P. citrella* suppression as well as duration of effectiveness by up to 1 week. This was likely because droplets containing only 0.8 μg of pheromone were not sufficiently competitive with females throughout the deployment period as the release rate dissipated below an attractive threshold sooner than the base formulation tested. The addition of permethrin to the formulation also proved necessary for efficacy given that the formulations containing the insecticide suppressed male activity 6 and 10.6× more than the formulations without permethrin (Figure 1). Both in our field and laboratory investigations, there was no evidence that the 6% permethrin loading in the MalEx formulation inhibited male *P. citrella* response to droplets containing pheromone. These results are congruent with several other studies showing that permethrin does not repel moths in pheromone-based attracticide formulations at loading rates ranging between 1 and 10% (Haynes et al., 1986; Evenden & McLaughlin, 2004b; Nansen & Phillips, 2004; Curkovic & Brunner, 2006; Evenden et al., 2008). At least one recent investigation of this attracticide formulation showed that the addition of permethrin did not increase efficacy against *G. molesta*, suggesting that mating disruption was the operative mechanism of control (Evenden & McLaughlin, 2004a). Depending on the dosage of pheromone released per dispenser, male orientation may be disrupted by either a non-competitive mechanism or a competitive mechanism without source contact. Either of these scenarios likely occurred in the investigation with *G. molesta* (Evenden & McLaughlin, 2004a). However, in our investigation, male *P. citrella* definitely approached and contacted droplets of MalEx, as documented by direct behavioral observations in the field. Some proportion of attracted males was suppressed by mating disruption without intoxication by permethrin as trap capture was reduced in plots treated with the insecticide-free formulation (Figure 1). At a deployment rate of 3 000 MalEx droplets ha^{-1}, only 8.7 g permethrin were deployed per ha of crop with the 6% formulation. However, this low dose of insecticide likely played a large role in suppression of *P. citrella* in the field as our laboratory investigation confirmed that 100% of males

contacting MalEx droplets, aged up to 35 days in the field, were killed within 24 h.

When the base formulation of MalEx was applied as 3 000 point sources ha^{-1}, the height of application did not influence efficacy of male *P. citrella* suppression within mature 4-m tall citrus trees (Figure 3). These data are congruent with a recent finding showing that male *P. citrella* are active throughout the tree canopy of mature citrus trees (Stelinski & Rogers, 2008) and do not exhibit a preferential response to pheromone sources at a specific height within the tree canopy as is exhibited by certain other moths, such as *C. pomonella* (Howell et al., 1990). Similar findings have also been reported with an attracticide formulation developed for *G. molesta*, where application height of LastCall droplets did not influence efficacy; mating of sentinel virgin females occurred only when placed in untreated portions of the tree canopy (Evenden & McLaughlin, 2004a). Equivalent efficacy of MalEx against *P. citrella* at each height tested within the canopies of mature trees should simplify deployment of the formulation by either hand or mechanized ground or aerial application methods.

Decreasing the rate of MalEx from 3 000 to 1 500 droplets ha^{-1} reduced efficacy as measured by both male *P. citrella* activity and larval infestation (Figure 1). These data are in contrast to those obtained with *G. molesta*, where efficacy with 1 500 and 3 000 droplets ha^{-1} was equivalent (Evenden & McLaughlin, 2004a). Although the highest rate tested (4 500 droplets ha^{-1}) did not result in statistically better efficacy than 3 000 droplets ha^{-1}, there was a noticeable trend for improved efficacy as the number of MalEx droplets per ha was increased. The profile of male *P. citrella* catch in traps as a function of point source density in Figure 2 is congruent with the conclusion that competitive attraction was an operative mechanism in this investigation (Miller et al., 2006). Thus, although 3 000 droplets ha^{-1} may prove effective under certain population densities, efficacy will be population density dependent and may require deployment of more droplets per ha in proportion to the size of the *P. citrella* population.

Continuous treatment of 0.5-ha blocks of citrus with MalEx against *P. citrella* over the course of 112 days reduced larval infestation of new leaf flush by 3.6–7.2×, depending on droplet application density. A similar reduction of *P. citrella* infestation was obtained with mating disruption, which deployed 3 g pheromone ha^{-1} (Stelinski et al., 2008). Reduced leaf infestation should result in reduced spread of citrus bacterial canker (Graham et al., 2004). Although this technology might benefit from companion use of selective insecticides to decrease *P. citrella* population densities, it also has the potential to reduce the number of required broad spectrum sprays for *P. citrella* management in both field and citrus nursery settings. Targeted deployment of insecticide with MalEx should decrease the harmful impact of current *P. citrella* management practices on the wide complex of natural enemies that are known to limit *P. citrella* population growth (Peña et al., 2002). Large-scale commercial use of this formulation will likely mandate the development of specialized mechanical applicators (Stelinski et al., 2007) for rapid deployment of material in the field.

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