

Investigating dormant-season application of pheromone in citrus to control overwintering and spring populations of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae)

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Abstract

BACKGROUND: The leafminer, *Phyllocnistis citrella* Stainton, reproduces on leaf flush during winter. Deployment of pheromone during winter could suppress moth populations in spring and summer more than a spring application alone. We tested the primary pheromone component of *P. citrella*, (Z,Z,E)-7,11,13-hexadecatrienal, released gradually over several months from elastomeric dispensers in a citrus grove in 6.4 ha main plots in winter and/or 3.2 ha subplots in spring (834 mg triene ha⁻¹) and evaluated moth catch and leaf mining.

RESULTS: After winter treatment, dispensers provided >85% disruption of male moth catch in traps for 37 weeks, and after spring treatment they provided >92% disruption for 26 weeks, but there was only a 12% reduction in leaf infestation in spring. Two applications were no better than only a single application in spring. Disruption of moth catch was weaker in treated plots where traps were placed high (3.1 m) rather than low (1.6 m) in the tree canopy.

CONCLUSION: Dispensers provided effective and persistent disruption of male catch in pheromone-baited monitoring traps but were minimally effective in reducing leaf infestation by *P. citrella*. Winter application of pheromone did not reduce leaf mining in spring compared with spring application alone. Tops of trees may have provided a refuge for mating.

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Keywords: citrus; leafminer; sex pheromone; mating disruption; preventive management

1 INTRODUCTION

The goal of preventive pest management is to suppress the density of a pest population well before it threatens to exceed an economic injury threshold.^{1,2} Preventive management may be accomplished by tactics that hinder pest establishment or expansion.² Several tactics are directed towards vulnerable stages of a pest's life cycle or during times of the year when pest populations are low. For example, the codling moth, *Cydia pomonella* (L.), is controlled most effectively by mating disruption when populations are low.^{3,4} In almond orchards, the navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae), is partially controlled by removal of infested mummy nuts during the dormant winter season.^{5–7} In Florida citrus, a single application of insecticide during the dormant season controls Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), for as long as 6 months.⁸ In European apple orchards, overwintering populations of several tortricid moth species are controlled with selective insecticides that restrain population growth, keeping them from rising to damaging numbers later in summer.⁹

The leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a worldwide pest of citrus (Rutaceae) whose larvae burrow into young leaves and create serpentine mines that damage leaves and stunt growth.^{10–12} The leafminer is multivoltine, with as many as 13 generations per year in Florida.¹¹ Small populations are

thought to overwinter without diapause, reproducing on the few leaf flushes available during winter.¹³ These small overwintering populations may be susceptible to preventive pest management tactics to decrease their subsequent proliferation in the spring and summer.

Mating disruption may provide such a tactic for controlling populations of *P. citrella* in citrus groves during winter and spring. Male moths of *P. citrella* are attracted to a blend of two pheromone components emitted by the female: (Z,Z,E)-7,11,13-hexadecatrienal (triene) and (Z,Z)-7,11-hexadecadienal (diene) released in a 3:1 ratio.¹⁴ Dispersing the natural pheromone blend or one of its components alone in citrus groves interferes with the ability of male moths to find traps baited with synthetic pheromone lures that

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act as surrogate females.^{15–17} Reduced capture of male moths in such traps provides a proxy measurement of mating disruption. Also, early investigations from small-plot trials (<30 trees plot⁻¹) suggested that damage to leaves by *P. citrella* could be reduced by application of mating disruption.¹⁵ Several methods have been used to release pheromone components of *P. citrella* into citrus groves, including elastomeric dispensers that gradually release the triene over a period of months.¹⁵ Release of the pheromone from dispensers follows classic first-order kinetics, with a half-life of 40 days and sustained release for 100 days in dispensers similar to those used in our study.¹⁸

The primary objective of this investigation was to evaluate the efficacy of applying mating disruption for *P. citrella* during the winter dormant season as a preventive pest management tactic. This was compared with replicated areas that received only spring applications of pheromone for mating disruption. Disruption of male moth catch in pheromone traps, incidence of leaf damage caused by mining and density of immature leafminers per leaf were used as measurements of efficacy. A secondary objective that emerged during the study was to evaluate trap catch disruption in treated plots at two heights within the tree canopy.

2 EXPERIMENTAL METHODS

2.1 Study site

Our study site consisted of two adjacent production blocks, each 1490 × 183 m, of mature ‘Flame’ grapefruit (*Citrus × paradisi* Macfad.) trees on Swingle rootstock in western St Lucie County, Florida (27° 28′ 18″ N, 80° 38′ 13″ W). Blocks were contiguous on the long (east to west) axis, separated by 16 m. Trees in each block were aligned north to south on double-row beds with 48 trees per row, 7.9 m between rows and 3.9 m between trees within rows.

We partitioned the two production blocks into treatments according to a 2 × 2 factorial design with split plots (Fig. 1) in four replicated blocks to test the main effect of triene applied in winter and the subplot effect of triene applied in spring. Main plots contained 22 rows × 96 trees (174 × 366 m) and were separated by buffers that were two rows (16 m) wide. Each main plot was partitioned into two subplots of 22 rows × 48 trees (174 × 183 m). We applied triene to main plots in winter (6–8 February) and to subplots in spring (24 April) at a rate of 834 mg ha⁻¹ by attaching experimental elastomeric dispensers that gradually release pheromone over a period of months (2.52 mg of triene; ISCA Technologies, Riverside, CA) to each tree in a treated area (331 ha⁻¹). Elastomeric dispensers were attached to branches 1–2 m within the canopy and 1–2 m above the ground using standard steel paper clips (Work Services, Wichita Falls, TX) that were punched through the dispensers and bent to form a hook. Trees were approximately 4 m tall and hedged to a width of 5 m. Therefore, dispensers were placed in the lower one-half to one-quarter of the tree canopy and within the inner two-thirds of the canopy.

2.2 Evaluation of trap catch disruption and leaf mines

To evaluate disruption of trap catch, we placed two Pherocon VI Delta traps in the center bed of each subplot, in the exterior canopy of the western row, 1–2 m above the ground, at points 61 and 122 m from ends of rows. We baited traps with ISCALure-citrella (ISCA Technologies), a pheromone lure that attracts male *P. citrella*, and we evaluated trap catch by counting the number of moths caught in a 177 cm² area on each sticky card trap liner.^{19,20} We averaged

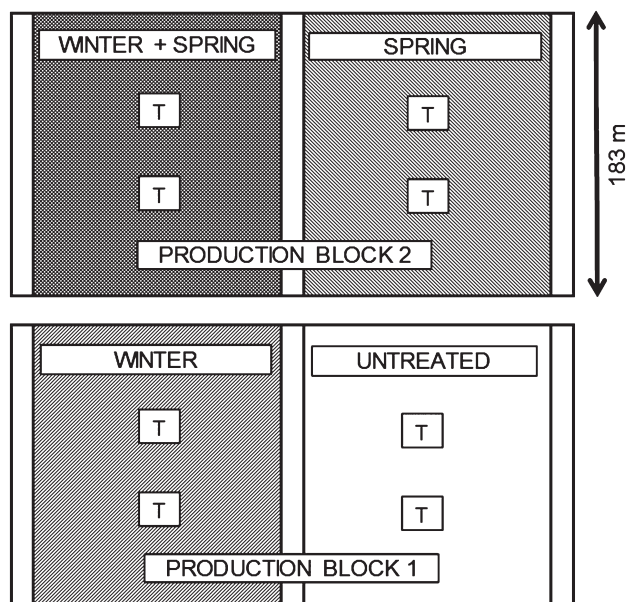


Figure 1. The study site in St Lucie County, Florida, showing one of four replicated statistical blocks as part of a 2 × 2 factorial design in split plots testing the main effect of triene applied during winter in 6.4 ha main plots and/or triene applied during spring in 3.2 ha subplots, and showing locations of pheromone-baited traps (T) to test disruption of trap catch at the low trap height (1.60 m).

trap catch within main plots before spring application of triene and across subplots after spring application of triene. Sticky trap liners were replaced every 3 weeks, and lures were replaced every 8 weeks. Traps were monitored from 16 January to 24 October 2013. To evaluate disruption of trap catch higher in the tree canopy, we placed additional pheromone-baited Pherocon VI Delta traps in the center bed of each subplot, in the exterior canopy of the western row, near treetops, 91 m from ends of rows. These traps were monitored from 20 August to 24 October.

We evaluated young foliage for infestation by *P. citrella* 4 times. Main plots were evaluated before (30 January) and after (26 March) application of triene during winter. Subplots were evaluated twice (5 June, 7 August) after application of triene in the spring. Evaluations were done by collecting samples of new flush shoots from the middle two rows of each main plot (40 shoots) or subplot (20 shoots). A flush shoot was eligible for sampling if it contained an actively growing meristem, typically colored light green. Shoots were broken off at their base, placed into sealable plastic bags and stored in a cooler. Samples were collected by walking along the entire length of a bed and selecting the new light-green-colored shoots from each side without immediate examination. In the laboratory, we measured the length of each shoot from base to meristem and counted the number of leaves per shoot, including all flattened leaves, which were typically longer than 0.5 cm. We examined each leaf under a dissecting microscope, counted the number of infested leaves and counted the number of active mines with live leafminers.

2.3 Statistical analysis

Counts of moths in main plots before and after application of triene during winter were analyzed by two-sample, two-tailed *t*-tests on each sample date. After application of triene in the spring, counts of moths were averaged across 26 weeks (183 days). Counts of moths in traps placed low and high in the canopy

were averaged across 9 weeks (65 days). These data, as well as proportion of infested shoots, proportion of infested leaves and number of leafminers per shoot, were analyzed by factorial ANOVA to test the main effect of application of triene in the winter, the subplot effect of application of triene in the spring and their interaction. Additionally, counts of moths in traps placed low and high within the canopy were analyzed by paired *t*-test, with low and high traps in each subplot representing a pair. Count data were square root ($x + 1$) transformed prior to analysis. Proportion data were arcsine square root transformed prior to ANOVA. For *t*-tests, we used a folded *F*-test to evaluate homogeneity of variance, and if the result was significant ($\alpha = 0.05$), we used a Satterthwaite approximation.²¹ Data are reported as original means \pm standard error of the mean. Data were analyzed using Statistix 9.²¹

3 RESULTS

3.1 Evaluation of moth catch in pheromone-baited traps

Moth counts (moths trap⁻¹ day⁻¹) were low (0.18 ± 0.03 , $n = 8$) during the 12 days (25 January to 6 February) preceding application of triene in winter (Fig. 2). Counts remained low (≤ 3) in untreated plots before rising sharply to 31 ± 3 (3 April, $n = 4$) and 63 ± 5 (24 April, $n = 4$) in April.

In plots treated with triene, counts fell to just 0.005 ± 0.005 by 19 February ($t = -8.5$, $P < 0.01$), 0.005 ± 0.003 by 8 March ($t = -16.7$, $P < 0.001$) and 0.06 ± 0.01 by 20 March ($t = -6.1$, $P < 0.01$), then remained significantly low at 1.0 ± 0.2 by 3 April ($t = -14.8$, $P < 0.001$) and 1.8 ± 0.4 by 24 April ($t = -17.4$, $P < 0.0001$).

After application of triene in the spring (24 April), the average number of moths caught during a period of 26 weeks (183 days) was: 2.9 ± 0.2 in plots treated during the winter only; 1.4 ± 0.3 in plots treated during the spring only; 1.2 ± 0.6 in plots treated during both the winter and spring; 29.5 ± 4.5 trap⁻¹ day⁻¹ in plots that were untreated (Fig. 2). There were significant differences in the main effect of treatment in the winter ($F_{1,3} = 93.8$, $P = 0.002$), the subplot effect of treatment in the spring ($F_{1,6} = 75.4$, $P = 0.0001$) and the winter \times spring interaction ($F_{1,6} = 43.4$, $P = 0.0006$) (Fig. 2). Disruption of trap catch remained $>90\%$ for 23 weeks (164 days) where plots were treated in the winter only (Table 1). Disruption

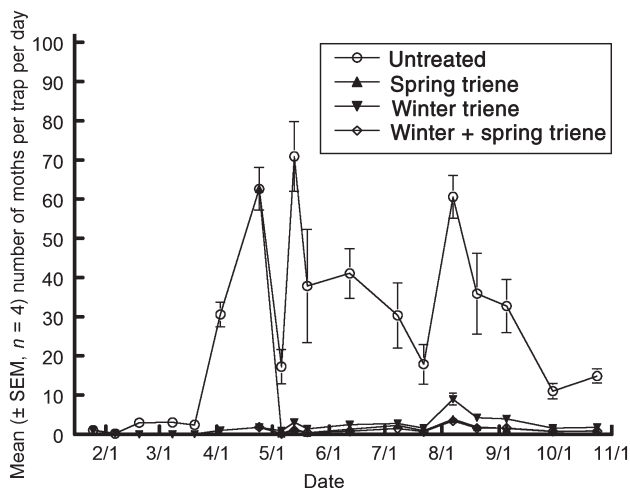


Figure 2. Number of *P. citrella* moths caught in delta traps baited with a pheromone lure in plots of grapefruit trees after application of triene (834 mg ha^{-1}) in winter (6.4 ha main plots) on 6–8 February and/or spring (834 mg ha^{-1} , 3.2 ha subplots) on 24 April, released from elastomeric dispensers over several months.

was similarly high ($>92\%$) for 26 weeks (183 days) where plots were treated in the spring only or the winter and spring ($t = -0.48$, $P = 0.65$).

Traps placed low in the tree canopy were $1.60 \pm 0.03 \text{ m}$ ($n = 32$) above the ground, whereas those placed high in the tree canopy were $3.14 \pm 0.09 \text{ m}$ ($n = 16$) above the ground. The average number of moths caught during a 9 week period (20 August to 24 October) in low traps (17.8 ± 3.7) and high traps (26.1 ± 3.6) did not differ in untreated plots ($t = -1.73$, $P = 0.18$). However, more moths were caught in high traps than in low traps where plots were treated in the winter only (12.5 ± 2.4 versus 2.2 ± 0.2 , $t = -3.96$, $P = 0.03$), the spring only (7.5 ± 1.1 versus 0.9 ± 0.1 , $t = -6.05$, $P = 0.009$) and the winter and spring (6.7 ± 1.3 versus 1.0 ± 0.4 , $t = -5.12$, $P = 0.014$) (Fig. 3). For high traps during that time period, the main effect of treatment in the winter was not significant ($F_{1,3} = 5.8$, $P = 0.10$), but the subplot effect of treatment in the spring ($F_{1,6} = 48.5$, $P < 0.001$) and the winter \times spring interaction ($F_{1,6} = 9.5$, $P = 0.02$) were significant. Disruption of trap catch was 26% lower in high versus low traps where plots were treated in the spring. Also, disruption was 20% lower in high versus low traps where plots were treated in the winter and spring (Fig. 3).

3.2 Evaluation of leaf mines

During the first evaluation for leafminer larvae (30 January), prior to application of triene in winter, shoots were $8.9 \pm 0.4 \text{ cm}$ ($n = 8$) long, and there were 10.2 ± 0.3 leaves per shoot. We found that $1.9 \pm 0.9\%$ of shoots were infested, $0.3 \pm 0.1\%$ of leaves were infested and there were 0.02 ± 0.01 active mines per ten leaves. On the second evaluation (26 March), 7 weeks after application of triene in winter, shoots were $11.1 \pm 0.2 \text{ cm}$ ($n = 8$) long, and there were 9.7 ± 0.1 leaves per shoot. No shoots were infested at this evaluation time. On the third evaluation (5 June), 6 weeks after application of triene in spring, shoots were $12.2 \pm 0.3 \text{ cm}$ ($n = 16$) long, and there were 12.3 ± 0.4 leaves per shoot. At that time, 51–70% of shoots were infested, 17–24% of leaves were infested and there were 2.8–4.8 active leaf mines per ten leaves; however, there were no differences in the main effect of winter treatment, the subplot effect of spring treatment or in their interaction (ANOVA, $P > 0.10$) (Table 1).

On the fourth evaluation (7 August), 15 weeks after application of triene in spring, shoots were $14.8 \pm 0.2 \text{ cm}$ ($n = 16$) long, and there were 13.6 ± 0.2 leaves per shoot. At that time, 76–85% of shoots were infested, 27–34% of leaves were infested and there were 4.4–5.4 active leaf mines per ten leaves (Table 2). There were no significant winter or spring treatment effects or interaction for percentage of infested shoots or numbers of leafminers. The percentage of infested leaves did not differ with winter treatment; however, the subplot effect of spring treatment and the interaction were significant, and there were 12% fewer infested leaves in spring-treated versus spring-untreated plots (Table 2). The significant interaction showed that the percentage of infested leaves was unchanged when winter-treated plots were treated again in spring, but infested leaves were reduced when winter-untreated plots were treated in spring.

4 DISCUSSION

Application of pheromone in winter and spring was not more effective at controlling *P. citrella* compared with application in spring alone. Disruption of trap catch for traps placed low in the canopy remained above 85% in treated plots for 37 weeks. The

Table 1. Percentage disruption (\pm SEM, $n = 4$) of catch of *P. citrella* in delta traps baited with a pheromone lure placed in blocks treated with triene (834 mg ha^{-1}) released over several months from elastomeric dispensers in winter and/or spring at two trap heights

Evaluation	% Disruption of <i>P. citrella</i>					
	Low traps (1.60 m)			High traps (3.14 m)		
	Winter ^a	Spring ^b	W + S ^c	Winter	Spring	W + S
19 February	99.8 \pm 0.2	–	–	–	–	–
3 March	99.8 \pm 0.1	–	–	–	–	–
20 March	97.6 \pm 0.4	–	–	–	–	–
3 April	96.9 \pm 0.7	–	–	–	–	–
24 April	97.1 \pm 0.6	–	–	–	–	–
6 May	94.9 \pm 0.5	98.9 \pm 0.8	98.9 \pm 0.8	–	–	–
13 May	95.7 \pm 1.5	98.2 \pm 1.2	98.6 \pm 0.6	–	–	–
20 May	96.3 \pm 0.7	98.9 \pm 0.5	98.6 \pm 0.2	–	–	–
12 June	94.1 \pm 0.9	96.9 \pm 1.1	98.1 \pm 1.1	–	–	–
8 July	90.8 \pm 1.4	92.7 \pm 2.7	95.2 \pm 3.0	–	–	–
22 July	91.3 \pm 0.8	95.0 \pm 1.5	96.1 \pm 2.2	–	–	–
7 August	85.2 \pm 2.5	93.7 \pm 1.0	94.4 \pm 2.8	–	–	–
20 August	88.2 \pm 1.8	94.9 \pm 1.0	95.7 \pm 2.2	–	–	–
5 September	88.0 \pm 1.1	95.6 \pm 0.1	95.0 \pm 2.6	52.0 \pm 10.9	79.1 \pm 3.1	74.7 \pm 5.6
30 September	85.6 \pm 1.8	93.4 \pm 1.2	93.1 \pm 1.5	44.5 \pm 8.0	59.3 \pm 7.4	72.7 \pm 3.5
24 October	87.9 \pm 2.3	94.3 \pm 1.7	94.5 \pm 3.2	58.0 \pm 11.0	65.6 \pm 12.0	74.4 \pm 15.5

^a Triene (834 mg ha^{-1}) applied to main plots (6.38 ha) in winter (6–8 February).

^b Triene (834 mg ha^{-1}) applied to subplots (3.19 ha) in spring (24 April).

^c W + S = winter and spring.

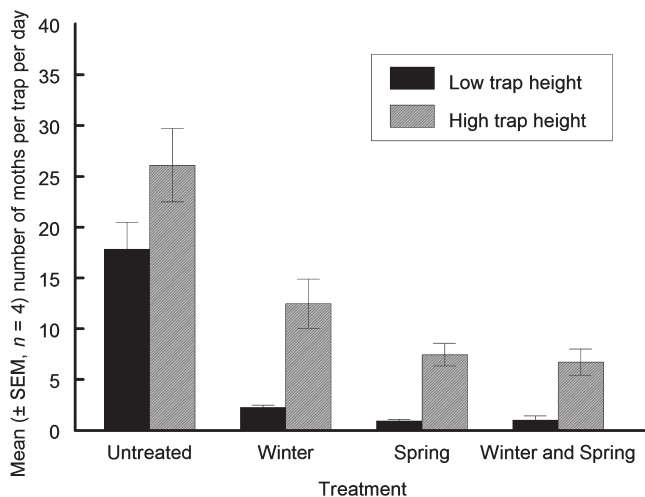


Figure 3. Number of *P. citrella* moths caught in pheromone-baited delta traps placed low (1.60 m) or high (3.14 m) in the canopy of grapefruit trees in subplots treated with triene (834 mg ha^{-1}) in winter and/or spring or untreated and monitored from 20 August to 28 October 2013.

level of trap catch disruption required to control this species in citrus groves is unknown, but the actual level of mating disruption in the grove may have been stronger than was suggested by trap catch because the dispensers, which act as surrogate females, are more attractive than virgin females of *P. citrella*.^{22,23} The findings of our study are similar to results of previous trials. In one study, two deployments of elastomeric septa loaded with blend ($1.33 \text{ mg septa}^{-1}$) and placed in Valencia orange trees at a density of $1100 \text{ septa ha}^{-1}$ (1.5 g AI ha^{-1} per deployment) provided nearly 100% trap catch disruption of *P. citrella* for 32 weeks and reduced infestation of flushes by 12%.¹⁵ Moreover, a density of

only $215 \text{ septa ha}^{-1}$ ($0.25 \text{ g AI ha}^{-1}$ per deployment) provided 70% trap catch disruption,¹⁵ which is lower than the pheromone rate investigated in this study ($0.75 \text{ g AI ha}^{-1}$ per application). In spite of the effective and persistent disruption of trap catch in the lower tree canopy in our study, leaf mining was only minimally reduced in treated plots at one evaluation; this was a 12% reduction in infested leaves per shoot 15 weeks after treatment during the spring.

Several factors may explain the high incidence and severity of leaf mining within treated plots in spite of strong disruption of trap catch lower within the canopy. Mining may have been a consequence of incomplete disruption in the upper tree canopy, where moths may have mated before laying eggs lower within the tree. In treated plots, we caught more moths in traps placed high (3.1 m) in the tree canopy than in traps placed low (1.6 m) in the canopy, but there was no such difference in untreated plots, indicating that disruption of trap catch was greater in the lower than in the higher traps within treated plots. Trap catch disruption may have been greater in low traps because the deployment of dispensers containing triene was at a height similar to where the low traps were placed. The triene may not rise sufficiently to the level of higher traps, or the pheromone may dissipate across that distance. The height of pheromone dispensers in citrus trees has been shown to be correlated with disruption of trap catch, which has led to a hypothesis that placing dispensers higher in the tree canopy would provide more effective disruption of mating throughout the tree.¹⁸ An alternative hypothesis to account for lack of disruption high in the canopy is that air currents passing through the upper canopy are less obstructed, which may create pockets of air that are free of pheromone, making it easier for moths to find traps and potential mates. Finally, moth behavior may be influenced by exposure to pheromone, and females may disperse to areas of lower pheromone concentration following autodetection.²⁴

Table 2. Incidence of infestation by *P. citrella* on young shoots and leaves of grapefruit trees and severity of infestation on leaves in blocks of grapefruit trees treated (+) with triene or untreated (–) in winter and spring, with the triene released from elastomeric dispensers over several months

Triene ^a		5 June			7 August		
Winter	Spring	% Shoots infested ^b	% Leaves infested	Leafminers per ten leaves	% Shoots infested	% Leaves infested ^c	Leafminers per ten leaves
–	–	52 ± 5	18 ± 3	3.0 ± 0.7	85 ± 6	34 ± 3	5.4 ± 0.5
+	–	51 ± 12	17 ± 5	2.8 ± 0.8	76 ± 3	29 ± 3	4.6 ± 0.4
–	+	70 ± 6	24 ± 2	4.8 ± 0.8	79 ± 5	27 ± 3	4.4 ± 0.8
+	+	53 ± 3	18 ± 2	3.1 ± 0.5	80 ± 5	29 ± 2	5.0 ± 0.2
ANOVA (F values)							
Winter		2.7	0.7	0.8	1.4	0.2	0
Spring		1.3	1.7	3.4	0.4	12.3*	1.2
Winter × spring		0.9	0.7	1.0	2.4	10.2*	4.9

^a Triene applied (834 mg ha⁻¹) to main plots (6.38 ha) on 8 February and to subplots (3.19 ha) on 24 April.
^b Data are means (± SEM, n = 4) for 20 flush shoots sampled from the middle bed in each subplot.
^c An *denotes F-values significant at α = 0.05.

Mining of leaves in treated plots may also be explained by movement of mated females into treated plots from neighboring areas. Indeed, we saw a disparity between moth flight and leaf mining early in our study that might suggest such movement. Moth flight was abundant in the early spring when leaf mines at our study site were still scarce. Moth flight was high by 3 April, with an abundance of 30 moths trap⁻¹ day⁻¹, but no mines were found a week earlier on 26 March. *P. citrella* may proliferate in neighboring groves in late winter and early spring, particularly within groves that are abandoned or less intensively managed, before migrating actively or passively to new sites. Male *P. citrella* have been captured in pheromone-baited traps as far as 6.4 km from a known source of host plants,²⁵ and movement of moths from a citrus grove across adjacent pastureland has been correlated with wind blowing from the grove, suggesting passive transport.²⁶ Such mobility suggests that mating disruption of *P. citrella* would likely benefit from an area-wide approach, as has been used for controlling *C. pomonella* in apple-growing regions of the world.²⁷ Moths collected in spring may also have emerged from higher portions of the tree canopy, as tops of trees were not sampled for mines in our study. However, mines of *P. citrella* are known to be more abundant in lower strata of trees as compared with the higher canopy.²⁸ Finally, some *P. citrella* may overwinter as adults. Ujiye²⁹ reported that *P. citrella* overwinters as adults within citrus trees in warmer citrus-growing regions of Japan and hypothesized that females undergo reproductive diapause, but no such diapause is known to occur in Florida.¹³

5 CONCLUSIONS

Pheromone application during the dormant winter season did not prove useful for reducing leaf mining in the spring as compared with a spring application alone. Mating disruption of *P. citrella* on a small scale is presently only minimally effective in reducing leaf infestation in citrus groves in Florida, in spite of effective disruption of male moth catch in monitoring traps. Mining of leaves in treated plots may have derived from inadequate disruption of mating in the upper canopy of trees or influx of mated females from neighboring areas, or because of dissipation of pheromone near edges of treated areas. Inadequate disruption in tops of trees could be mitigated by placing pheromone dispensers higher within canopies of trees. Dissipation of pheromone owing to edge effects

could be mitigated by increasing the size of the treated area or by increasing the density of dispensers near edges of treated areas. Insecticide applications near borders might also improve control of this species within pheromone-treated areas. Our results corroborate previous investigations and suggest that management of *P. citrella* with mating disruption on a small scale may be ineffectual. Our results point to the likely need for large-scale area-wide treatments for effective mating disruption of *P. citrella*. We have initiated such area-wide applications and will report on these investigations in the near future.

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REFERENCES

- 1 Rabb RL, A sharp focus on insect populations and pest management from a wide-area view. *Bull Entomol Soc Am* **24**:55–61 (1978).
- 2 Pedigo LP, Closing the gap between IPM theory and practice *J Agric Entomol* **12**:171–181 (1995).
- 3 Barnes MM, Millar JG, Kirsch PA and Hawks DC, Codling moth (Lepidoptera: Tortricidae) control by dissemination of synthetic female sex pheromone. *J Econ Entomol* **85**:1274–1277 (1992).
- 4 Gut LJ and Brunner JF, Pheromone-based management of codling moth (Lepidoptera: Tortricidae) in Washington apple orchards. *J Agric Entomol* **15**:387–406 (1998).
- 5 Zalom FG, Weakley C, Hendricks LC, Bentley WJ, Barnett WW and Connell JH, Cultural management of the navel orangeworm by winter sanitation. *Calif Agric March–April*:**28** (1984).
- 6 Connell JH, Leading edge of plant protection for almond. *Horttechnology* **12**:619–622 (2002).
- 7 Eilers EJ and Klein A, Landscape context and management effects on an important insect pest and its natural enemies in almond. *Biol Control* **51**:388–394 (2009).

- 8 Qureshi JA and Stansly PA, Dormant season foliar sprays of broad-spectrum insecticides: an effective component of integrated management for *Diaphorina citri* (Hemiptera: Psyllidae) in citrus orchards. *Crop Prot* **29**:860–866 (2000).
- 9 Bloomers LHM, Integrated pest management in European apple orchards. *Annu Rev Entomol* **39**:213–241 (1994).
- 10 Achor DS, Browning H and Albrigo LG, Anatomical and histochemical effects on feeding by citrus leafminer larvae (*Phyllocnistis citrella* Stainton) in citrus leaves. *J Am Soc Hort Sci* **122**:829–836 (1997).
- 11 Heppner JB and Fasulo TR, Citrus leafminer, *Phyllocnistis citrella* Stainton (Insecta: Lepidoptera: Phyllocnistinae). *University of Florida Publication EENY038*. [Online]. University of Florida. Available: <https://edis.ifas.ufl.edu/pdffiles/IN/IN16500.pdf> [22 July 2015].
- 12 Richardson ML, Westbrook CJ, Hall DG, Stover E and Duan YP, Abundance of citrus leafminer larvae on citrus and citrus-related germplasm. *HortScience* **46**:1260–1264 (2011).
- 13 Lim UT and Hoy MA, Overwintering of the citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), without diapause in Florida. *Fla Entomol* **89**:361–366 (2006).
- 14 Lapointe SL, Hall DG, Murata Y, Parra-Pedrazzoli AL, Bento JMS, Vilela EF *et al.*, Field evaluation of a synthetic female sex pheromone for the leafmining moth *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Florida citrus. *Fla Entomol* **89**:274–276 (2006).
- 15 Stelinski LL, Miller JR and Rogers ME, Mating disruption of citrus leafminer mediated by a noncompetitive mechanism at a remarkably low pheromone release rate. *J Chem Ecol* **34**:1107–1113 (2008).
- 16 Lapointe SL, Stelinski LL, Evens TJ, Niedz RP, Hall DG and Mafra-Neto A, Sensory imbalance as mechanism of orientation disruption in the leafminer *Phyllocnistis citrella*: elucidation by multivariate geometric designs and response surface models. *J Chem Ecol* **35**:896–903 (2009).
- 17 Stelinski LL, Lapointe SL and Meyer WL, Season-long mating disruption of citrus leafminer, *Phyllocnistis citrella* Stainton, with an emulsified wax formulation of pheromone. *J Appl Entomol* **134**:512–520 (2010).
- 18 Lapointe SL, Keathley CP, Stelinski LL, Urrutia WH and Mafra-Neto A, Disruption of the leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in citrus: effect of blend and placement height, longevity of disruption and emission profile of a new dispenser. *Fla Entomol* **98**:742–748 (2015).
- 19 Lapointe SL and Leal WS, Describing seasonal phenology of the leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) with pheromone lures: controlling for lure degradation. *Fla Entomol* **90**:710–714 (2007).
- 20 Stelinski LL and Rogers ME, Factors affecting captures of male citrus leafminer, *Phyllocnistis citrella* Stainton, in pheromone-baited traps. *J Appl Entomol* **132**:143–150 (2008).
- 21 *Statistix Version 90: User's Manual*. Analytical Software, Tallahassee, FL (2008).
- 22 Leal WS, Parra-Pedrazzoli AL, Cossé AA, Murata Y, Bento JMS and Vilela EF, Identification, synthesis, and field evaluation of the sex pheromone from the citrus leafminer, *Phyllocnistis citrella*. *J Chem Ecol* **32**:155–168 (2006).
- 23 Lapointe SL, Stelinski LL, Keathley CP and Mafra-Neto A, Intentional coverage gaps reduce cost of mating disruption for *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in citrus. *J Econ Entomol* **107**:718–726 (2014).
- 24 Palanaswamy P and Seabrook WD, Behavioral responses of the female eastern spruce budworm *Choristoneura fumiferana* (Lepidoptera, Tortricidae) to the sex pheromone of her own species. *J Chem Ecol* **4**:649–655 (1978).
- 25 Kawahara AY, Jones M, Jai QJ, Lapointe SL and Stansly PA, A synthetic pheromone for *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) attracts multiple leafminer species. *Fla Entomol* **96**:1213–1216.
- 26 Keathley CP and Lapointe SL, Movement of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) from a grove to traps and sentinel plants in adjacent land. *Fla Entomol* **97**:291–294 (2014).
- 27 Witzgall P, Stelinski L, Gut L and Thompson D, Codling moth management and chemical ecology. *Annu Rev Entomol* **53**:503–522 (2008).
- 28 Tsagkarakis AE, Perdakis DC and Lykouressis DP, Seasonal abundance and within tree spatial distribution of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). *Entomol Gen* **33**:165–174 (2011).
- 29 Ujiye T, Biology and control of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), in Japan. *Jpn Agr Res Q* **34**:167–173 (2000).