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FIELD EVALUATION OF A SYNTHETIC FEMALE SEX PHEROMONE FOR THE LEAFMINING MOTH *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE) IN FLORIDA CITRUS

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The leafmining moth, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was discovered in southern Florida in 1993 (Heppner 1993) and has since spread to all Florida citrus-growing counties and the states of Alabama, Louisiana, Texas in 1994, and California (Gil 1999) and Hawaii in 2000 (Nagamine & Heu 2003). Damage includes loss of photosynthetic capacity from mining, stunting and malformation of leaves, and potential damage from increased susceptibility of leafminer-damaged leaves to the citrus canker pathogen (Bergamin-Filho et al. 2000; Cook 1988).

Ando et al. (1985) found attraction in Japanese populations of *P. citrella* to traps baited with (*Z*,*Z*)-7,11-hexadecadienal. Attempts to show attraction of this material to populations in other countries were not successful (Sant'ana et al. 2003). Leal et al. (2006) found the three active compounds (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal [*Z*7*Z*11*E*13-16Ald], (*Z*,*Z*)-7,11-hexadecadienal [*Z*7*Z*11-16Ald], and (*Z*)-7-hexadecenal [*Z*7-16Ald] by electroantennograms (EAG) from female pheromone gland extracts of a Brazilian population of *P. citrella* in a ratio of 30:10:1, respectively (Fig. 1). They also demonstrated that traps baited with a mixture of the two major constituents caught more males than traps baited with virgin female *P. citrella*.

Here we report the results from two field trials. The first documents attraction to a binary lure consisting of the two major EAG-active components. In the second trial, we deployed a factorial design to determine the influence of trap height in a mature citrus grove on trap catch and the relative attraction of a binary and a tertiary lure. For the first trial, six traps (Pherocon 1C Wing Trap, Trecé, Inc., Adair, OK) were deployed in a citrus grove at the experimental farm of the U.S. Horticultural Research Laboratory, Ft. Pierce, FL. Three traps were baited with rubber septa impregnated with a binary pheromone mixture consisting of *Z*7*Z*11*E*13-16Ald (96% pure) and *Z*7*Z*11-16Ald (98% pure) in a ratio of 3:1, and three traps were left without lures as controls. Septa were loaded with 50 µg of the major and 17 µg of the minor compound in 100 µl hexane per septum. Traps were randomly assigned to orange trees (approximately 2 m tall) within a section of 6 rows of 29 trees/row naturally infested with citrus leafminer. Traps were rotated daily between trees and the adhesive cards were removed daily and examined for the presence of *P. citrella*. Traps were deployed and counted for 3 d in August, 2005.

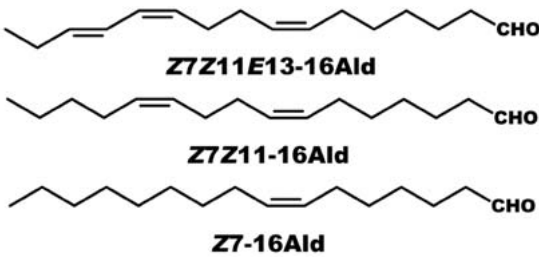

Fig. 1. Chemical structure of three semiochemicals isolated from pheromone gland extracts of the leafmining moth *P. citrella*.

For the second trial, a factoral design ($3 \times 3 \times 6$) was used to investigate the effect of trap height and pheromone lure composition on trap catch over 6 d. Traps were deployed at heights of 1.3, 1.7, and 2.0 m within the same section of orange trees used in Trial 1. Traps were baited with septa impregnated with either the binary mixture as in Trial 1, a tertiary blend consisting of *Z*7*Z*11*E*13-16Ald and *Z*7*Z*11-16Ald and *Z*7-16Ald in a ratio of 30:10:1, or control septa impregnated with 100 µl hexane. Three replications were used for a total of 27 traps per d and 162 (3 height × 3 lures × 6 d) total traps counted over 6 d. Traps were randomly assigned to trees within the section. Spacing of the trees was 4.6 m within rows

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and 7.6 m between rows. Adjacent trees were avoided to maintain a minimum of 9 m between traps. Traps with corresponding lures and fresh adhesive cards were randomly re-assigned to trees daily. Traps were deployed and counted for 6 d in August, 2005. During hurricane Katrina, traps and lures were removed; lures were stored at -80°C and re-deployed 3 d later. High levels of parasitization of leafminer larvae in the field, presumably by *Ageniaspis citricola* (Hymenoptera: Encyrtidae), interfered with our attempts to rear virgin female leafminers to compare with the synthetic lures.

Counts were made of the entire card (23 × 28 cm). We transformed data to normalize residuals before analysis by using natural log (x + 1). All tests of significance were based on transformed data. Untransformed means are presented. Data were analyzed by ANOVA. When significant differences were indicated by the F statistic at $\alpha = 0.05$, means were separated by Fisher's protected least significant difference (LSD) (Abacus Concepts 1996).

No *P. citrella* adults were captured in the unbaited traps with the exception of one adult on d 3. A total of 391 *P. citrella* was captured in traps baited with the binary lure. There was no significant effect of d ($F = 2.73$; $df = 2, 12$; $P = 0.106$). More *P. citrella* were captured in traps baited with the binary lure ($F = 9.92$; $df = 1, 16$; $P < 0.0001$). The binary lure attracted a mean (± SEM) of 43.4 ± 7.8 compared with 0.4 ± 0.3 adults/trap/d in the control traps.

In the second trial, a total of 6 adult *P. citrella* over 6 d was found in traps baited without pheromone. Traps baited with the binary mixture caught a total of 508 *P. citrella* and the tertiary mixture caught 605 *P. citrella*. There was no effect of trap height on trap catch ($F = 1.02$; $df = 2, 108$; $P = 0.366$). There was a significant effect of d on trap catch ($F = 6.90$; $df = 5, 144$; $P < 0.0001$) (Table 1) with a significant lure × day interaction ($F = 1.96$; $df = 10, 144$; $P = 0.042$). The effect of lure was significant ($F = 358.52$; $df = 2, 144$; $P < 0.0001$). There was no significant difference between the binary and tertiary lures for any of the days tested except for d 1 when there was a significant height effect ($F = 3.90$; $df = 2, 18$; $P = 0.039$) and a significant interaction between height and lure ($F = 3.90$; $df = 4, 18$; $P = 0.019$). This was due to a higher capture in the traps baited with the tertiary lure at 2 m (39.3 ± 7.4) compared with 13.7 ± 2.0 at 1.3 m and 17.7 ± 7.4 at 0.7 m. On all subsequent days there was no effect of height, no significant difference between binary and tertiary lures, nor was there a significant interaction between height and lure ($\alpha = 0.05$) (Table 1).

TABLE 1. MEAN (± SEM, $n = 3$) NUMBER OF *P. CITRELLA* ADULTS CAPTURED IN TRAPS BAITED WITH RUBBER SEPTA IMPREGNATED WITH A BINARY LURE, A TERTIARY LURE, OR UNBAITED (CONTROL) OVER 6 D AT FT. PIERCE, FL.

Day	Lure	Catch
1	Control	0.2 + 0.1 a
	Binary	15.0 + 2.4 b
	Tertiary	23.6 + 4.8 c
2	Control	0.2 + 0.1 a
	Binary	6.6 + 1.5 b
	Tertiary	7.0 + 1.5 b
3	Control	0.1 + 0.1 a
	Binary	8.4 + 1.1 b
	Tertiary	12.3 + 3.7 b
4	Control	0.0 + 0.0 a
	Binary	7.3 + 1.8 b
	Tertiary	6.9 + 1.6 b
5	Control	0.0 + 0.0 a
	Binary	10.4 + 1.6 b
	Tertiary	9.9 + 1.9 b
6	Control	0.1 + 0.1 a
	Binary	8.7 + 1.2 b
	Tertiary	7.6 + 1.2 b

Means grouped by d followed by the same letter are not significantly different ($\alpha = 0.05$, Fisher's protected LSD).

SUMMARY

Traps baited with a binary mixture in the ratio of 30:10 of two EAG-active compounds, (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal and (*Z*,*Z*)-7,11-hexadecadienal, attracted significantly more moths of the leafmining moth *P. citrella* compared with unbaited traps in a Florida citrus grove. The addition of a third EAG-active compound, (*Z*)-7-hexadecenal, did not increase trap catch. Trap height, at 0.7, 1.3, and 2 m did not significantly affect daily trap catch on 5 of 6 d. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that also may be suitable.

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