

# Synthetic sex pheromone of citrus leafminer in Brazilian citrus groves

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**Abstract** – The objective of this work was to determine the best conditions of use of the synthetic sex pheromone of *Phyllocnistis citrella* Stainton for monitoring this species in citrus groves in northeastern Brazil. Pheromone doses (0.0, 0.1, 1, 10 and 100 µg) and longevity (1, 15, 29, 43 and 57-day-old lures) and trap height (0.5, 1.5 and 2.5 m), color (green, red, and white) and model influence on *P. citrella* males capture were evaluated. The doses of 10 and 100 µg of the synthetic sex pheromone – a 3:1 blend of (Z,Z,E)-7,11,13-hexadecatrienal and (Z,Z)-7,11-hexadecadienal – attracted the greatest number of *P. citrella* males. Traps baited with these two both dosages continued to capture *P. citrella* males at a comparable rate for over eight weeks in citrus groves. Although there was no significant decrease in activity of both dosages until 57 days of exposure to the environment, the higher dose, as time passed, attracted significantly more *P. citrella* males than the lower dose. There were no significant differences in male capture in traps with synthetic sex pheromone placed at 1.5 and 2.5 m height, wich had the better results. Trap color and model did not affect male capture.

**Index terms:** *Phyllocnistis citrella*, (Z,Z)-7,11-hexadecadienal, (Z,Z,E)-7,11,13-hexadecatrienal, monitoring.

## Feromônio sexual sintético do minador-dos-citros em pomares brasileiros de citros

**Resumo** – O objetivo deste trabalho foi determinar as melhores condições de uso do feromônio sexual sintético de *Phyllocnistis citrella* Stainton para o monitoramento dessa espécie em pomares de citros no Nordeste do Brasil. Foram avaliados: doses (0,0, 0,1, 1, 10 e 100 µg), duração (1, 15, 29, 43 e 57 dias de exposição do feromônio sexual sintético), altura de instalação (0,5, 1,5 e 2,5 m), cor (verde, vermelha e branca) e modelo de armadilha na captura de machos de *P. citrella*. As doses de 10 e 100 µg do feromônio sintético – proporção de 3:1 da mistura de Z,Z,E-7,11,13-hexadecatrienal e (Z,Z)-7,11-hexadecadienal – atraíram o maior número de machos de *P. citrella*. Armadilhas contendo a mistura feromonal nessas duas doses capturaram machos de *P. citrella* por mais de oito semanas em pomares de citros. Embora nenhuma das duas doses tenha sofrido decréscimo na atividade por até 57 dias de exposição ao ambiente, com o passar do tempo a maior dose atraiu significativamente mais machos de *P. citrella* que a menor dose. Não houve diferença significativa na captura de machos nas armadilhas com o feromônio sexual instaladas a 1,5 e 2,5 m de altura, as quais apresentaram os melhores resultados. O modelo e a cor das armadilhas não afetaram a captura dos machos.

**Termos para indexação:** *Phyllocnistis citrella*, (Z,Z)-7,11-hexadecadienal, (Z,Z,E)-7,11,13-hexadecatrienal, monitoramento.

## Introduction

The citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) was originally described in India (Stainton, 1856), but is now established in five continents (Heppner, 1993; Argov & Rössler, 1996; Prates et al., 1996). In Brazil, *P. citrella* was discovered in the State of São Paulo in 1996 (Prates et al., 1996)

and spread rapidly to all citrus-growing municipalities in Brazil (Chiaradia & Milanez, 1997).

Damages caused by the citrus leafminer include loss of photosynthetic capacity from mining, stunting, and malformation of leaves. Besides those damages, the larval mining increases the susceptibility of trees to the citrus canker disease, caused by *Xanthomonas axonopodis* pv. *citri* (Cook, 1988; Bergamin-Filho

et al., 2000). Chagas et al. (2001) observed that infection by *X. axonopodis* pv. *citri* in leafminer-damaged citrus leaves was up to eleven times greater when compared with infection in undamaged leaves.

There appear to be differences in response to pheromone compounds between populations of *P. citrella*. Ando et al. (1985) and Van Vang et al. (2008) reported attraction in Japanese populations of *P. citrella* to traps baited with the single compound (Z,Z)-7,11-hexadecadienal. However, attempts to show attraction of *P. citrella* populations to this pheromone in other countries were not successful (Sant'Ana et al., 2003). Leal et al. (2006) detected three active compounds from female pheromone gland extracts of a Brazilian population of *P. citrella*: (Z,Z,E)-7,11,13-hexadecatrienal [Z7Z11E13-16Ald], (Z,Z)-7,11-hexadecadienal [Z7Z11-16Ald], and (Z)-7-hexadecenal [Z7-16Ald] in a ratio of 30:10:1. They also demonstrated that traps baited with a mixture of the two major constituents captured more males than traps baited with virgin female *P. citrella*. Moreira et al. (2006) identified Z7Z11-16Ald and Z7Z11E13-16Ald by Gas Chromatography-Electroantennography (GC-EAG) as critical components of the pheromone and reported that the isomeric (Z,Z,Z)-7,11,13-hexadecatrienal inhibited attraction to the binary blend. In contrast to the Japanese population, Moreira et al. (2006) showed that field populations of *P. citrella* in California, USA, did not respond to either of the major compounds alone. Lapointe et al. (2006) and Van Vang et al. (2008) showed that 3:1 binary mixtures of Z7Z11E13-16Ald and Z7Z11-16Ald were attractive to field populations in Florida, USA and in Vietnam, respectively.

In Brazil, the recently identified sex pheromone (Leal et al., 2006; Moreira et al., 2006) had never been tested in field conditions, and many factors affecting *P. citrella* capture in pheromone-baited traps must be determined with the intent of developing effective monitoring.

The objective of this study was to determine the best conditions of use of the synthetic sex pheromone of *P. citrella* for monitoring this species in citrus groves in northeastern Brazil.

## Materials and Methods

The experiments were conducted at Itaporanga d'Ajuda, SE, Brazil (10°57'30"S; 37°28'11"W), in a

citrus (*Citrus sinensis* L. Osbeck) variety Pera Rio crop with a 4x7 m spacing, from July to November 2007.

For the determination of the optimal pheromone dose for male trapping, 0.0, 0.1, 1, 10 and 100 µg of the *P. citrella* synthetic pheromone – a 3:1 blend of (Z,Z,E)-7,11,13-hexadecatrienal and (Z,Z)-7,11-hexadecadienal (Fuji Flavor Co., Japan) (Leal et al., 2006; Moreira et al., 2006) – were tested in a two-year-old 'Pera Rio' citrus grove. The pheromone aliquots were incorporated into slow-release devices made of ES fiber (Ethylene-Propylene Side By Side, Chisso Co. Ltd, Japan) and coated with polyethylene film. Pheromone dispensers were attached inside diamond-model sticky traps (CPO Ltda., Brazil), and placed at a height of approximately 1.5 m (middle part of the plants), at the edge of the citrus tree canopy.

The experimental design was completely randomized, with five treatments and four replicates, in a total of 24 traps. The traps were placed at a 40-m distance from each other. The number of male moths captured per trap was observed 24 hours after deployment. Data were transformed to log (x + 1) to normalize variance and were analyzed by ANOVA. Means were compared by Tukey's test, at 5% probability.

To estimate optimal trap height, traps baited with 10 µg of a 3:1 blend of the synthetic pheromone (dose that provided great male captures, observed in the previous experiment) were installed in a four-year-old 'Pera Rio' citrus grove at 0.5, 1.5 and 2.5 m heights, which corresponded to the lower, middle, and upper parts of the plants.

The experimental design was completely randomized, with three treatments and four replicates, in a total of 12 traps. The number of male moths captured per trap was inspected 24 hours after deployment. Data were transformed to log (x + 1), submitted to analysis of variance and the means were compared by Tukey's test at 5% probability after a significant ANOVA.

To assess the effect of trap model and color on males capture, traps were deployed approximately 1.5 m high (middle part of the plants) and baited with 10 µg of the synthetic pheromone blend. Yellow traps were not used because they are known to attract a large number of nontarget insects (Silveira Neto et al., 1976).

The experimental design was completely randomized, in a 3x3 factorial arrangement with three trap models (delta, diamond, and cylinder) and three trap colors

(green, red, and white), with four replicates. Traps were placed in two-year-old 'Pera Rio' trees with a 40-m spacing between one another, and were inspected 24 hours after deployment. The number of male moths captured per trap was transformed to root square ( $x + 8$ ) and compared by Tukey's test at 5% probability after a significant ANOVA.

To determine the longevity of the pheromone dispenser, sex pheromone lures with 10 or 100  $\mu\text{g}$  (best doses previously determined) of the 3:1 synthetic pheromone blend were aged by unsealing the pheromone dispensers (lures) at different dates prior to field experiments, and maintaining them under field conditions until tested. Sets of four lures each were unsealed on May 17, May 31, June 14, June 28, and July 12, 2007. This allowed the comparison of 1, 15, 29, 43 and 57-day-old lures at the same time. Diamond sticky traps baited with these matured devices were deployed on July 12, 2007 in a two-year-old 'Pera Rio' citrus grove. Traps with lures were placed at 1.5 m height with 40 m spacing between traps.

The experimental design was completely randomized, in a 2x5 factorial arrangement with two doses (10 and 100  $\mu\text{g}$ ) and five lure longevities (1, 15, 29, 43 and 57 days), with four replicates (each batch of devices contained four replicates). The number of male moths captured per trap was inspected 24 hours after deployment. Data were transformed to root square ( $x + 3$ ) and the mean was compared by Tukey's test at 5% probability after a significant ANOVA.

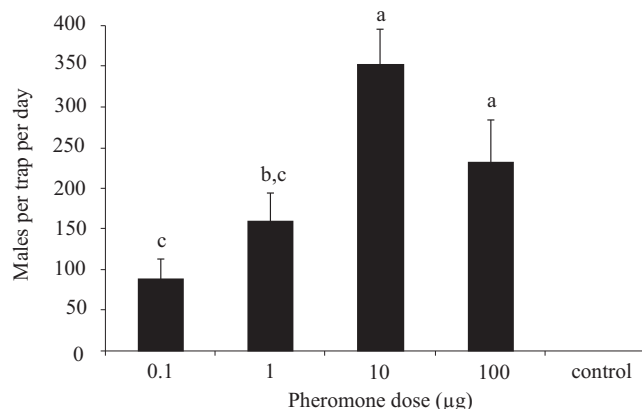
## Results and Discussion

The pheromone doses showed significant effect in male capture. The greatest male captures were observed in traps baited with 10 and 100  $\mu\text{g}$ , with no significant differences between them (Figure 1). This result demonstrates that the previously identified sex pheromone of the citrus leafminer [(Z,Z,E)-7,11,13-hexadecatrienal, (Z,Z)-7,11-hexadecadienal, 3:1] (Leal et al., 2006; Moreira et al., 2006) is an effective tool for monitoring *P. citrella* field populations. Lapointe et al. (2006) also observed that traps baited with the same pheromone blend attracted significantly more *P. citrella* male moths when compared with unbaited traps in a citrus grove in Florida, USA.

There was no significant difference between the number of males caught in traps placed at 1.5 m

(401.75 $\pm$ 47.64) and 2.5 m (393.38 $\pm$ 40.18), but this number was significantly greater when compared with traps placed at 0.5 m (224.75 $\pm$ 32.68). For practical applications, it is recommended that traps are placed in the middle part of citrus trees. Stelinski & Rogers (2008), using the same pheromone of the present study in mature citrus trees, observed that the male capture was highest at 2 m above ground level within the tree canopy and at 0.6 m above ground level on the perimeter of the tree canopy. Lapointe et al. (2006) observed that trap height at 0.7, 1.3 and 2 m did not significantly affect the daily rate of male catch in traps.

Male catches using all of the tested pheromone-baited trap models and colors were similar. The main effects of trap model ( $p = 0.52$ ), trap color ( $p = 0.92$ ) and the interaction between model and color ( $p = 0.80$ ) were not significant. The mean was 163.19 $\pm$ 22.32 males per trap per day. White traps are more practical because they are readily identified in the field due to their contrast with the green citrus leaves, and also because a white background facilitates insect count. Stelinski & Rogers (2008) observed that Delta-style traps baited with the same pheromone blend used in the present study were highly effective in catching *P. citrella* males in orange trees in Lake Alfred, Florida, USA.



**Figure 1.** Number of *Phyllocnistis citrella* males captured in traps baited with synthetic sex pheromone, (Z,Z,E)-7,11,13-hexadecatrienal and (Z,Z)-7,11-hexadecadienal (3:1), with different doses (0, 0.1, 1.0, 10 and 100  $\mu\text{g}$ ), after 24 hours of deployment, in citrus (*Citrus sinensis* L. Osbeck) variety Pera Rio trees. Means $\pm$ SE followed by equal letters do not differ significantly by Tukey's test at 5% probability.

Traps baited with 10 and 100 µg of the synthetic sex pheromone did not lose the attractiveness as time passed, since both doses captured a similar number of males in each evaluation from the first ( $p = 0,42$ ) until the 57<sup>th</sup> day. Although there was no significant decrease in activity of both dosages during 57 days of exposure to the environment, the mean total number of males caught in the higher dose was greater ( $46.85 \pm 2.75$ ) than in the lower dose ( $25.90 \pm 6.86$ ) ( $p = 0,0020$ ). Lapointe & Leal (2007), testing the same pheromone blend in a Florida citrus grove, observed a 50% loss of lure attractiveness 50 days after lure deployment and a 90% loss 137 days after deployment.

The monitoring of citrus leafminer field population with synthetic sex pheromone traps can rationalize its control, reducing production costs and environmental impacts. Given the importance of the *P. citrella* pest worldwide, the synthetic sex pheromone might be useful in citrus seedling nurseries for the production of pathogen-free seedlings, especially regarding *Xanthomonas axonopodis* pv. *citri* bacterium. It can also be used to demarcate areas subject to tighter citrus canker inspections, taking into account that areas with higher *P. citrella* catches are more likely to have the disease.

### Conclusions

1. The ideal dose of *Phyllocnistis citrella* synthetic sex pheromone, (Z,Z,E)-7,11,13-hexadecatrienal and (Z,Z)-7,11-hexadecadienal (3:1), for monitoring this species in citrus groves is 100 µg.

2. Pheromone lure with 100 µg of the synthetic pheromone blend is active for a period of 57 days.

3. Trap placement in the middle and upper parts of the citrus plants present better results regarding *P. citrella* capture efficiency.

4. The models and colors of the traps do not interfere with *P. citrella* capture efficiency.

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