A Synthetic Pheromone for Phyllocnistis citrella (Lepidoptera: Gracillariidae) Attracts Multiple Leafminer Species

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The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was first detected in Florida in 1993 and quickly spread throughout the state (Heppner 1995). Leaf mining causes a decline in leaf photosynthesis and increased susceptibility to citrus canker (Graham et al. 2004; Gottwald et al. 2007). Incidence and severity of *P. citrella* (CLM) damage have increased in Florida recently, possibly due to resurgence of leafminer populations resulting from intensified spray programs directed against the Asian citrus psyllid (*Diaphorina citri*). Consequent increases in canker are especially notable in young trees and susceptible varieties such as grapefruit and early season oranges (Dewdney & Graham 2012).

One of the most common methods to monitor CLM abundance is to use a pheromone lure to attract the adult moth. Ando et al. (1985) reported attraction of male *P. citrella* in Japan to (*Z*,*Z*-*E*)-7,11-hexadecatrienal (diene). Elsewhere, including Florida, the diene alone did not attract males (Jacas & Peña 2002). Leal et al. (2006) identified two additional aldehydes from pheromone glands of female *P. citrella* from Brazil, (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal (triene) and (*Z*)-7-hexadecenal (monoene), in a ratio of 30:10:1 triene:diene:monoene. However, inclusion of the monoene in a ternary blend did not increase trap catch of males in Florida compared with a 3:1 triene:diene blend (Lapointe et al. 2006). Moreira et al. (2006) also identified the triene and diene compounds from *P. citrella* populations in California, USA, and reported an optimal ratio of 3:1 triene: diene to attract males. The 3:1 ratio was confirmed as optimal in Florida (Lapointe et al. 2009). Recently, the presence of a congeneric leafminer native to Florida, *P. insignis* (Frey & Boll), was observed in sticky traps baited with *P. citrella* lures loaded with the 3:1 blend (Keathley et al. 2013). Here we provide evidence that the 3:1 blend of (*Z*,*Z*,*E*)-7,11,13-hexadecatrienal and (*Z*,*Z*)-7,11-hexadecadienal attracts multiple unrelated and genetically distinct species of *Phyllocnistis* in southern Florida.
studies (e.g., Kawahara et al. 2011; Kawahara & Rubinoff 2012; De Prins & Kawahara 2012), the final dataset was subject to a maximum likelihood phylogenetic analysis. We conducted 1000 best tree searches and 1000 bootstrap replicates, applying a GTR+I+G substitution model with a random starting tree in the program GARLI (Zwickl 2006). All sequences are available in GenBank (www.ncbi.nlm.nih.gov).

Phyllocnistis citrella, P. insignis, P. vitegenella and 2 unidentified congeners were obtained from traps baited with ISCAlure Citrella™ lures at locations in Collier, Hendry, Lee, and St. Lucie Counties. Based on COI sequence data, at least 5 genetically divergent Phyllocnistis species were attracted to the lure. Two unidentified species (circles 4 and 5, Fig. 1) share a separate origin from P. citrella and constitute a genetically distinct group from P. citrella and P. insignis. Because this preliminary study was conducted at a limited number of sites in Central Florida, it is possible that additional species in the genus might be attracted to the major components of the P. citrella sex pheromone. Species in the genus often appear morphologically similar based on wing pattern (Kawahara et al. 2009; De Prins & Kawahara 2009; Davis & Wagner 2011), therefore it is likely that past surveys overlooked the presence of multiple Phyllocnistis species in traps. The morphological similarity of these leaf miner species implies that estimates of P. citrella infestation in citrus groves might be influenced by non-P. citrella species, and therefore caution is required when making estimates on damage based on the number of moths attracted to lures.

This study was a preliminary investigation of Phyllocnistis species attracted to pheromone lures. We sequenced only one gene for this initial screening, and we plan to sequence additional samples and loci in the future. A study that utilizes a combination of morphology and multiple molecular markers (e.g., Mitter et al. 2010) will be necessary to conclusively determine the true identities of the clusters we observed from the COI barcode region alone.

**SUMMARY**

The citrus leafminer, *Phyllocnistis citrella* Stainton, is a major pest of citrus throughout the world. The larval stage of the moth mines leaves and reduces photosynthesis and increases the incidence and severity of citrus canker disease. A lure comprised of 2 aldehyde compounds isolated from pheromone glands of female *P. citrella* is widely used to monitor field populations. We conducted a preliminary morphological and molecular analysis to examine candidate species of *Phyllocnistis* that are attracted to pheromone lures containing the 2 major components of *P. citrella*. Our results demonstrated that several species of *Phyllocnistis*, including *P. insignis* and *P. vitegenella*, are attracted to the 2 major pheromone components of *P. citrella*. 

**Key Words:** citrus leafminer, citrus canker, lure, molecular phylogeny, Phyllocnistinae

**RESUMEN**

El minador de los cítricos, *Phyllocnistis citrella* Stainton, es una de las plagas principales de los cítricos en el mundo, ya que causa daño a las hojas por sus galerías, que reduce la capacidad...
Las fotosintética de las hojas y aumenta la inciden-
cia y la gravedad de la enfermedad del cáncer de
cítricos. Un señuelo hecho de 2 compuestos de
aldehído aislado de las glándulas de feromonas
de la hembra de *P. citrella* es ampliamente utili-
zado para monitorear las poblaciones en el cam-
po. Se realizó un análisis molecular preliminar
utilizando la región de código de barras del gen mitocondrial citocromo c oxidasa I (COI)
para examinar especies candidatas de *Phyllocnisi-
tis* que son atraídas a la feromona señuelo que
contiene los 2 componentes principales de la fe-omona sexual de *P. citrella*. Nuestros resultados
demostraron que los grupos genéticamente diver-
gentes de los individuos son atraídos a los 2 prin-
cipales componentes de la feromona de *P. citrella*.

Fig. 1. Preliminary maximum likelihood molecular phylogeny of *P. citrella* and relatives based on COI alone. The 5 tentative clusters collected from traps baited with a 3:1 blend of *(Z,Z,E)-7,11,13-hexadecatrienial and *(Z,Z)-7,11-
hexadecadienal are indicated with thick lines, with circles denoting their origin. Numbers above or beside branches are bootstrap values.
Palabras Clave: minador de los cítricos, el cancro cítrico, señuelo, filogenia molecular, Phyllocnistinae

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REFERENCES CITED


Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. The University of Texas at Austin, Ph.D. dissertation.