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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*)-7,11-hexadecadienal (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 mononene 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.

Recent research in Florida has focused on the use of pheromone traps to determine optimal application timing of insecticides, efficacy of insecticides, optimal trap density, correlation between CLM damage and adult trap counts, and number of CLM generations per year (Jones, unpublished). The traps (Great Lakes IPM, IPS-G004) containing an insecticide dispenser (Vaportape™ II, Great Lakes IPM, HC-8500-25) and a lure loaded with a 3:1 blend of (*Z,Z,E*)-7,11,13-hexadecatrienal and (*Z,Z*)-7,11-hexadecadienal (IT203 ISCAlure-Citrella, ISCA Technologies, Riverside, California) were monitored weekly between Feb and Nov (2011 and 2012), and bi-weekly from Jan through Dec. Trap locations included large commercial citrus groves in Collier, Hendry, Lee, and St. Lucie Counties, Florida, the U.S. Horticultural Research Laboratory at Ft. Pierce, Florida and a 35,000 acre unmanaged natural area in Okaloacoochee Slough State Forest (Table 1). The latter is a habitat characterized by marsh, cypress, wet prairie, pine flatwoods, oak hammocks, and oak-palm hammocks straddling southwestern Hendry and northwestern Collier counties. A single trap was centrally located in the upper canopy of trees of each selected citrus grove, and 12 traps were placed in the slough at varying distances (1.6, 3.2, 4.8, and 6.4 km) from known citrus. Pheromone lures were replaced every 6 to 8 weeks.

Moths were separated by morpho type and specimens of each type were sequenced for the 658 bp "barcode region" of the mitochondrial cytochrome *c* oxidase I (*CO1*) gene following our published techniques (e.g., Kawahara et al. 2013; Kawahara & Rubinoff 2013; Rubinoff et al. 2012). The COI sequences generated were combined with known *Phyllocnistis* COI sequences from GenBank (www.ncbi.org) and BOLD (www.boldsystems.org). We included 4 gracillariid outgroups, *Acrocercops astericola*, *Cameraria ohridella*, *Phyllonorycter acerifoliella* and *P. junoniella*. Sequences were assembled, edited, and aligned using Geneious 5.4 (Biomatters). We applied the "Geneious Alignment" option with default settings and manually checked the alignment. As we have done previously in our

TABLE 1. THE NUMBER OF EACH MORPH (A-F) COLLECTED, AS DETERMINED BY PHYSICAL EXAMINATION FOR EACH LOCATION AT COMMERCIAL CITRUS GROVES IN COLLIER, HENDRY, LEE, AND ST. LUCIE COUNTIES, FLORIDA (2011-2012). GROVES 2 AND 3 ARE COMMERCIAL ORANGE. "*PHYLLOCNISTIS* SP." REFERS TO UNIDENTIFIED *PHYLLOCNISTIS* SPECIES THAT FALL INTO CLADES 4 AND 5 IN FIG. 1. "OTHER" REFERS TO NON-*PHYLLOCNISTIS* SPECIES.

Year	Location	No.	*P. citrella*	*P. insignis*	*P. vitegenella*	*Phyllocnistis* sp.	Other
2011	OK Slough* 1.6 km	5	1	3			1
	OK Slough 3.2 km	38	13	1	23	1	
	OK Slough 4.8 km	33	16	2	14		1
	OK Slough 6.4 km	15	8	1	6		
2012	OK Slough 1.6 km	5	5				
	OK Slough 3.2 km	69	66	3			
	OK Slough 4.8 km	135	116		18	1	
	OK Slough 6.4 km	27	20	2	3	2	
	Grove 2 (Lee Co.)	46	31		15		
	Grove 3 (Collier Co.)	48	40		8		
	Total	421	316	12	87	4	2

*Okaloacoochee slough state forest is located near Felda in southwestern Florida and straddling southwestern Hendry and northwestern Collier counties.

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* 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 the *P. citrella* sex pheromone. 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

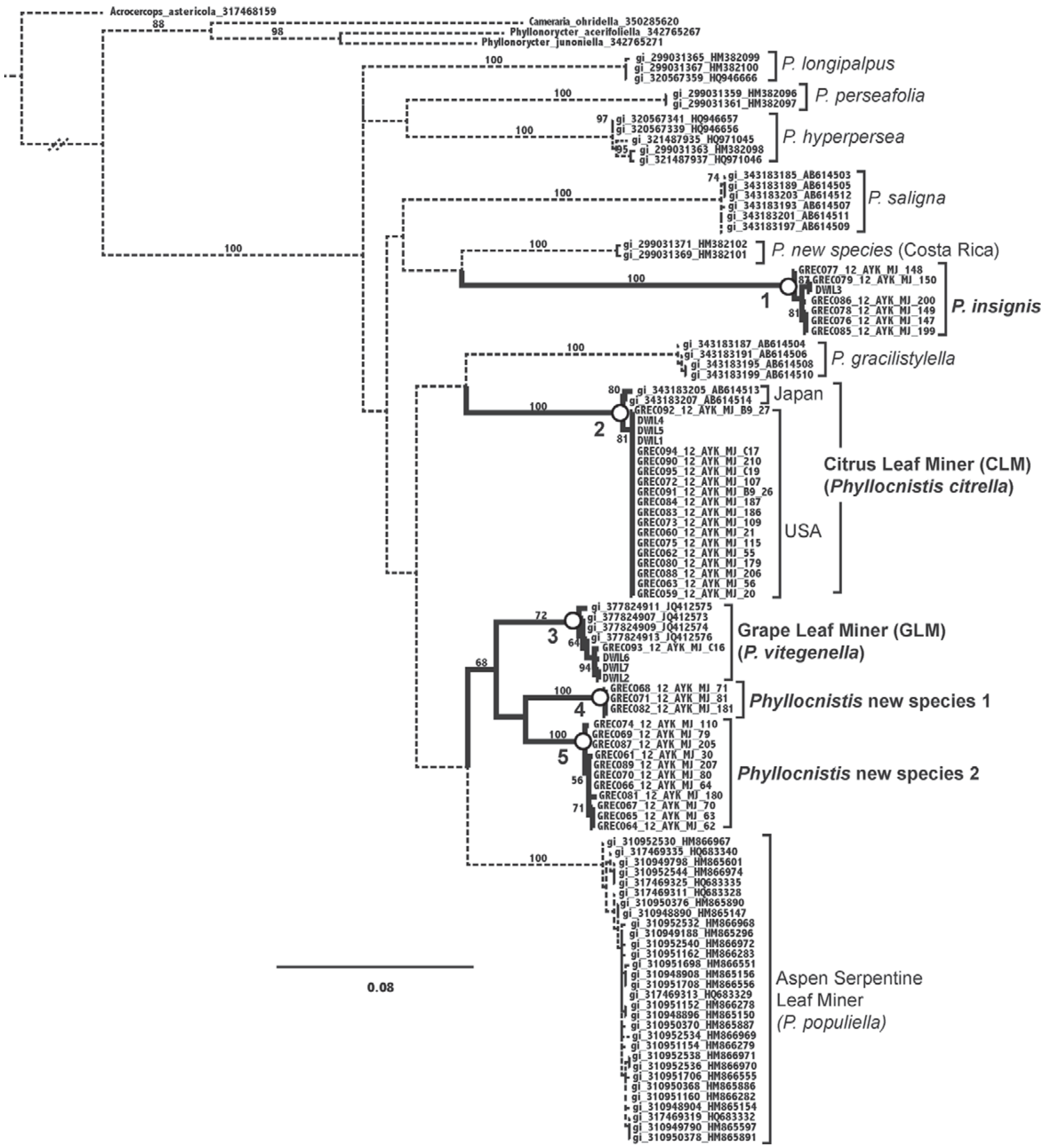

Fig. 1. Preliminary maximum likelihood molecular phylogeny of *P. citrella* and relatives based on COI alone. The 5 tentative clusters collected from traps baited with a 3:1 blend of (*Z,Z,E*)-7,11,13-hexadecatrienal and (*Z,Z*)-7,11-hexadecadienal are indicated with thick lines, with circles denoting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

fotosintética de las hojas y aumenta la incidencia y la gravedad de la enfermedad del cáncro de los cítricos. Un señuelo hecho de 2 compuestos de aldehído aislado de las glándulas de feromonas de la hembra de *P. citrella* es ampliamente utilizado para monitorear las poblaciones en el campo. Se realizó un análisis molecular preliminar utilizando la región de código de barras" 658 pb del gen mitocondrial citocromo *c* oxidasa I (*CO1*) para examinar especies candidatas de *Phyllocnistis* que son atraídas a la feromona señuelo que contiene los 2 componentes principales de la feromona sexual de *P. citrella*. Nuestros resultados demostraron que los grupos genéticamente divergentes de los individuos son atraídos a los 2 principales componentes de la feromona de *P. citrella*.

Palabras Clave: minador de los cítricos, el cancro cítrico, señuelo, filogenia molecular, Phyllocnistinae

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