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CITRUS LEAFMINER, *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE), AND NATURAL ENEMY DYNAMICS IN CENTRAL FLORIDA DURING 2005

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ABSTRACT

After the citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), invaded Florida in 1993, the endoparasitoid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) was introduced in 1994 in a classical biological control program. Subsequent to its establishment, only limited information has been obtained regarding the seasonal abundance of *A. citricola* and its host in central Florida citrus groves. During 2005, we monitored replicated plots treated with oil or imidacloprid once on 23 Jun 2005, along with untreated control trees, in a Polk County commercial Valencia orange grove on a weekly basis when tender new growth (= flush) was available. As expected, CLM abundance in the early spring flush was nearly undetectable due to the lack of suitable flush during winter when CLM populations decline nearly to zero. Also as expected, *A. citricola* was not found during this time. During the second flush (Jun through Jul) CLM populations increased and *A. citricola* appeared, parasitizing up to 39% of the pupae in the untreated controls and up to 33% in the blocks treated with oil. Imidacloprid did not significantly reduce the number of CLM larvae but did reduce Asian citrus psyllid, *Diaphorina citri* Kuwayama, nymphal densities. Peak abundance of the CLM occurred during the third flush cycle on 5 Oct from trees treated once with oil, with a mean (SD) of 1.3 (0.8) CLM mines per leaf. Parasitism by *A. citricola* increased through the season, peaking at 56% of the CLM that had pupated prior to the 16 and 23 Nov samples in the untreated control trees and at 37% in the oil-treated trees; *A. citricola* was not found in imidacloprid-treated trees on those dates. During the growing season, a high proportion (up to 100% in some samples) of the CLM mines were empty, presumably due to predation. The data confirmed, for the first time, that *A. citricola* is an important natural enemy of those CLM larvae that escaped predation in this citrus-growing area in Florida. Nymphs of the Asian citrus psyllid were significantly reduced for 3 weeks after the imidacloprid treatment. However, shoots on trees treated with imidacloprid were significantly shorter than shoots on untreated trees and the number of shoots produced in imidacloprid-treated trees was reduced, raising concerns that imidacloprid might affect growth of citrus flush. Brown citrus aphids were nearly absent throughout the growing season.

Key Words: citrus leafminer, *Ageniaspis citricola*, population dynamics, imidacloprid, oil, phytotoxicity

RESUMEN

Después de que el minador de la hoja de los cítricos (MHC), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), invadió el estado de la Florida en 1993, el endoparasitoide *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) fue introducido durante 1994 para un programa de control biológico clásico. Subsiguiente a su establecimiento, información obtenida en cuanto a la abundancia estacional de *A. citricola* y sus hospederos en huertos de cítricos en Florida central fue muy limitada. Durante el año del 2005, nosotros realizamos un monitoreo en parcelas replicadas tratadas con aceite o imidacloprid por una vez en el 23 de junio de 2005, incluidos con árboles no tratados para control, en un huerto comercial de naranjas "Valencia" en el condado de Polk todo ello revisado semanalmente cuando los brotes de nuevas hojas fueron disponibles. Como fue esperado, la abundancia de MHC en los brotes de nuevas hojas en el principio de la primavera fue casi no detectable debido a la falta de brotes apropiados durante el invierno cuando la población de MHC bajó a casi cero. A su vez, como era de esperar, *A. citricola* no fue encontrado durante este tiempo. Durante el segundo brote de hojas (junio a julio) la población de MHC aumentó y *A. citricola* apareció, parasitizando hasta 39% de la pupas en los bloques no tratados de control y hasta 33% de los bloques tratados con aceite. Imidacloprid no redujó significativamente el número de larvas de MHC pero si redujó la densidad de las ninfas del psila de cítrico Asiático, *Diaphorina citri* Kuwayama. El pico de la abundancia de MHC ocurrió durante el ciclo del tercer brote de las hojas nuevas en el 5 de octubre en árboles tratados una vez con aceite, con un promedio (DS) de 1.3 (0.8) minas de MHC por hoja. El parasitismo por *A. citricola* aumentó a travez de la estación, llegando a un climax de 56% de los MHC que han empupado antes de las muestras de 16 y 23 de noviembre en árboles no tratados y 37% en árboles tratados con aceite; *A. citricola* no fue encontrado en árboles tratados con imidacloprid en estas fechas. Durante la estación de crecimiento, una alta proporción (hasta 100% en algunas muestras) de las minas de MHC fueron vacias, propuestamente debido a la depredación. Los datos confirmaron, por primera vez, que *A. citricola* es un enemigo natural importante de las larvas de de MHC que escapan a los depredadores en esta área en Florida donde se siembra cítricos. Las ninfas del psila de cítrico Asiático fueron reducidas significativamente por 3 semanas después del tratamiento con imidaloprid. Sin embargo, los brotes en los árboles tratados con imidacloprid fueron significativamente mas cortos que en los árboles no tratados y el número de los brotes producidos en árboles tratados con imidacloprid fue reducido, aumentando la preocupación de que el imidacloprid posiblemente puede estar afectando el crecimiento de los brotes de los cítricos. El áfido pardo de los cítricos [*Toxoptera citricida*] durante la estación de crecimiento estuvo casi ausente.

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was discovered in Florida in May of 1993 and quickly spread through >800,000 acres of citrus, attacking tender new growth (= flush) and, occasionally, fruits and stems when densities were particularly high (Heppner 1993). Shortly after the invasion, native parasitoids (primarily eulophids) attacked this pest, as well as generalist predators (Browning & Peña 1995; Peña et al. 1996; Evans 1999; Amalin et al. 1996), but growers still considered CLM densities too high and treated both mature and young groves multiple times per season (Heppner 1995; Knapp et al. 1996). The host-specific endoparasitoid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) was imported from Australia (Neale et al. 1995) and first released in Florida in May 1994 in a classical biological control program (Hoy & Nguyen 1994a; 1997; Smith & Hoy 1995). A subsequent importation of *A. citricola* from Taiwan also was released (Hoy et al. 2000). Although the Australian (which was originally from Thailand) and Taiwan populations appeared morphologically identical, subsequent molecular studies indicated that they were, in fact, cryptic species (Hoy et al. 2000; Alvarez & Hoy 2002). Both populations were released in Florida, but a subsequent analysis failed to show that the Taiwan population had established (Alvarez 2000).

Establishment and spread of *A. citricola*, presumably the Australian population, in Florida was rapid and high rates of parasitism of CLM pupae were observed (Hoy & Nguyen 1994a; 1994b; 1997; Hoy et al. 1995; 1997; Pomerinke & Stansly 1998; Amalin et al. 1996; 2002). However, after establishment and dispersal were documented, funding for monitoring the phenology and dynamics of the CLM and *A. citricola* was unavailable because the CLM 'problem' appeared to have been solved, at least temporarily. As a result, information about the abundance and phenology of *A. citricola* in Florida's citrus groves remained anecdotal. Concerns about CLM population densities in Florida resurfaced during the citrus canker eradication program, because mines produced by CLM larvae allow the canker bacteria access to ideal growing conditions (Sohi & Sandhu 1968; Chagas et al. 2001; Gottwald et al. 2001; Graham et al. 1996; Christiano et al. 2007). In addition, *A. citricola* appeared less effective during 2000-2002 than in previous years because Florida was undergoing a drought and this parasitoid performs poorly when relative humidity is low (Yoder & Hoy 1998). Despite the fact that natural enemies cannot eliminate all CLM in a grove and even a single CLM can cause damage to foliage that increases the susceptibility of a tree to canker infection, consideration was given to importing additional parasitoids of the CLM, with the goal of further reducing CLM densities and, hopefully, canker incidence. *Semielacher petiolatus* Girault (Hymenoptera: Eulophidae) was imported and evaluated in quarantine, but not released because the potential risk of disrupting biological control by *A. citricola* was considered higher than the potential benefit of establishing *S. petiolatus* in Florida (Lim & Hoy 2005: Lim et al. 2006).

During 2005, we monitored a commercial citrus block in central Florida (Polk County) near Haines City each week during the major flush cycles to evaluate the phenology and relative abundance of the CLM and *A. citricola* in Valencia oranges that were untreated or treated with oil or with imidacloprid. We also evaluated additional mortality factors of the CLM. Relative abundances of the Asian citrus psyllid, *Diaphorina citri* Kuwayama, and the brown citrus aphid, *Toxoptera citricida* Kirkaldy, on the flush also were recorded.

Materials and Methods

Plots were established in a Valencia orange grove near Haines City, Florida (GPS coordinates: N 28°03.656, W 081°34.937). The trees were 4-5 years old and spaced 7.3 m apart between the rows and 3 m within the rows. During 2004, the year prior to this study, the only pesticide applied in this grove was petroleum oil (470 weight oil, (Petro-Canada, Calgary, Alberta), which was applied 3 times (May, Jul, and Sep) at a rate of 7 gal/acre (26.5 L/ 0.4 ha), which should not have had significant negative effects on *A. citricola* in the grove because oil has little residual toxicity (Villanueva-Jimenez & Hoy 1998). Trees were drip irrigated as needed and fertilized in 2004 with Nutri 5 at 1 qt/acre (0.95 L/0.4 ha) and with 3 Key-Plex foliar sprays at a rate of 2 qt/acre (1.89 L/0.4 ha) each. During 2005, a foliar application of potassium nitrate (N:P:K at a rate of 13.75-0-46) was added on 18 Oct 2005 and an organic amendment from poultry houses was added at a rate of 1000 lbs/acre (453.6 kg/0.4 ha) on 1 Sep 2005. During 2005 no sprays, other than those required by the experiment, were applied.

Randomized complete blocks with 4 treatments and 3 replicates of each treatment were set up in Mar, with each replicate consisting of 3 adjacent rows of 10 trees each with 3 buffer rows between each of the treatments. Trees were left untreated between replicates to reduce any effect of spray drift. Out of 30 trees in an experimental unit, 6 central, uniform and healthy trees were labeled and 4 young shoots per tree were collected, when present, at weekly intervals throughout the 2005 growing season from Mar until the end of Nov. Each week the percentage of terminals having new flush was estimated to determine the flushing patterns.

The 4 planned treatments consisted of (A) untreated control, (B) 3 sprays at 6-week intervals starting in Jun when the flush was about 3 cm in length with petroleum oil 455 (Petro-Canada, Calgary, Alberta) at 2% (20 mL/L of water), (C) 1 application of imidacloprid (Provado 1.6 F, Bayer CropScience, North Carolina) at the lowest recommended foliar application rate of 10 oz/acre (295 mL/0.4 ha) when the flush was 3 cm long, and (D) 2 sprays of petroleum oil 455 in weeks 1 and 3 of the Jun flush cycle at 2%. Treatment B was planned because many growers were using this spray schedule and Treatment D was planned to determine whether 2 treatments during the Jun flush cycle (second cycle) would allow *A. citricola* to 'catch up' with the citrus leafminer population and eliminate the need for additional sprays. Because populations of the citrus leafminer and its host-specific parasitoid *A. citricola* decline to very low levels during the winter in Florida when very little tender new growth is available to support reproduction of the leafminer (Lim & Hoy 2006), populations of both species may be nearly undetectable during the first flush cycle in Feb or Mar (Villanueva-Jimenez et al. 2000). Treatment C was considered the standard to which the 2 oil treatments would be compared. However, only 1 application of oil was applied on 23 Jun to treatments B, C, and D because so few CLM were present during Jun through Nov that additional sprays could not be justified.

When flush was present, 4 shoots longer than 0.5 cm were collected from each of 6 trees in each replicate and placed in a labeled plastic bag containing a paper towel to soak up any moisture that could cause the leaves to begin to rot prior to scoring. Plastic bags were placed in an ice chest with ice packs and shipped to the University of Florida, Department of Entomology and Nematology in Gainesville by FedEx overnight delivery.

Samples were scored with the aid of a dissecting microscope. Shoot length and numbers of leaves per shoot were recorded, as well as the number of CLM mines (>0.5 cm) per leaf, the number of CLM larvae in mines that were alive, parasitized, absent, or dead. Larvae missing from the mines were assumed to be dead from predation if no pupal chambers were associated with the mine. Pupal chambers were opened and the number of CLM pupae that were alive, parasitized by *A. citricola* (including number of *A. citricola* pupae) or by other parasitoids, or dead due to unknown causes was recorded. Relative abundance per shoot of Asian citrus psyllids was reported as 0 = none, 1 = 1-20, 2 = 21-50, 3 = 51-80, and 4 = >80 and the relative abundance of brown citrus aphids was reported as 0 = none, 1 = <10, 2 = 10-50, and 3 = >50. Data were analyzed by ANOVA and means separated by Fisher's least significant difference (LSD) test, based on 5% level of significance (SAS Institute, Cary, NC, USA).

Weather data were obtained from the nearest weather station at the Citrus Research and Education Center, Lake Alfred (http://fawn.ifas.ufl.edu/scripts/reportrequest.asp), and averaged each week over the experiment; the weather station is approximately 12.8 km from the experimental site.

Results and Discussion

During 2005, the study trees produced flush suitable for CLM, Asian citrus psyllid, and brown citrus aphid populations 3 times (Fig. 1). The first flush cycle began by 22 Feb and ended by 18 Apr; the second cycle began around 13 Jun and ended around 2 Aug, and the third began 27 Sep and ended by 30 Nov 2005. Tender new growth suitable for oviposition by CLM females was present during the first week of each flush cycle and the flush continued to grow and harden off during the subsequent 5 or 6 weeks. The proportion of tree branches that were flushing during the first 2 flush cycles was not different, but there were fewer ($F = 3.26$, $df = 3$, $P = 0.025$) flushes in the imidacloprid-treated trees during the third flush cycle. The average weekly temperature (°C), relative humidity (% RH), and rainfall (cm) during the trial are shown in Fig. 2.

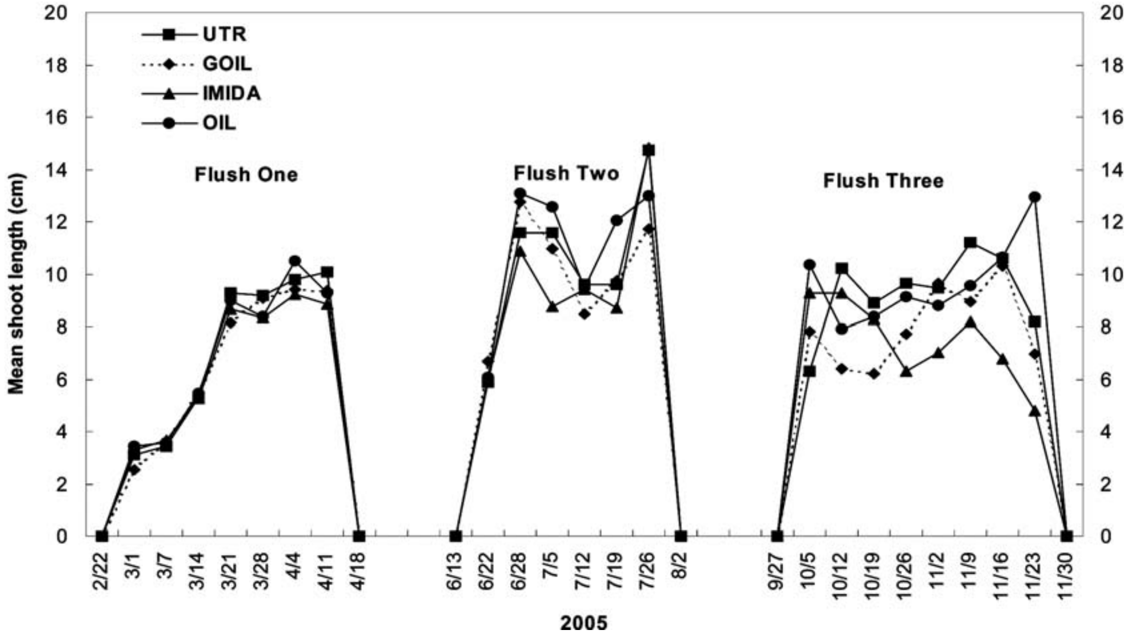

Fig. 1. Mean shoot length (cm) of flushes from a Valencia orange grove near Haines City, Florida in 2005.

First Flush

During the 7 weeks of the first flush cycle there were no differences among the treatments in timing of flush (Fig. 1). Likewise, there were no significant differences in shoot length among the treatments when the data were analyzed weekly, or over the entire flush cycle (Fig. 1) ($F = 0.11$, $df = 3$, $P = 0.95$). No CLM mines were observed except for 1 sample date (1 Mar), when an average of 0.01 (SD ± 0.12) mines/leaf were observed in the untreated control trees (Table 1). No *A. citricola* or other mortality factors were observed during this flush.

Densities of Asian citrus psyllid nymphs during the first flush were in categories 0 and 1, with category 0 indicating no psyllids and category 1 indicating 1-20 psyllids/shoot were present (Table 2). During weeks 1 through 5, there were no significant differences in psyllid densities among the 4 treatments when data were analyzed on a weekly basis ($P = 0.20$ to 0.95). When psyllid densities were analyzed over the entire flush cycle, no significant differences occurred among treatments ($F = 0.03$, $df = 3$, $P = 0.99$). No brown citrus aphids were observed during this flush.

Second Flush

On 22 Jun, prior to the application of sprays, very low numbers of CLM mines (0.04 to 0.07 CLM mines/leaf) were present (Table 1). The proportion of living CLM larvae in the mines on 22 Jun ranged from 79% (in the block to be treated with imidacloprid) to 100% (all other blocks) (Fig. 3). The mortality observed in the imidacloprid-treated trees was probably due to predation, because the mines were empty and no pupal chambers were present (Fig. 4).

After the sprays were applied on 23 Jun, the number of mines remained low throughout the subsequent 5 weeks, with mean densities during week 6 ranging from 0.10 to 0.15 CLM mines/leaf (Table 1). After the trees in treatments B, C, and D were sprayed with oil or imidacloprid, the number of living CLM larvae dropped to 12% in the 28 Jun sample and to zero in the 5 Jul sample in the imidacloprid-treated trees while 28, 36, and 16% of the larvae in the control and 2 oil treatments remained alive, respectively (Fig. 3). However, the number of live CLM larvae in the 4 treatments was not significantly different over the entire second flush when densities were combined over the 6 weeks ($F = 1.25$, $df = 3$, $P = 0.37$). This indicates that neither oil nor imidacloprid significantly reduced CLM larval feeding damage during this flush cycle. The maximum densities of CLM mines in this flush occurred on 28 Jun and ranged from 0.05 (0.03) CLM mines per leaf to 0.29 (0.12) mines per leaf (Table 1). Thus, densities of CLM larvae remained low during the entire second flush.

The proportion of CLM mines that were empty, presumably due to predation, during the second flush ranged from 21 to 97% (Fig. 4). There were no differences ($F = 1.58$, $df = 3$, $P = 0.29$) in the proportion of empty mines among the 4 treatments over the flush cycle, suggesting that the

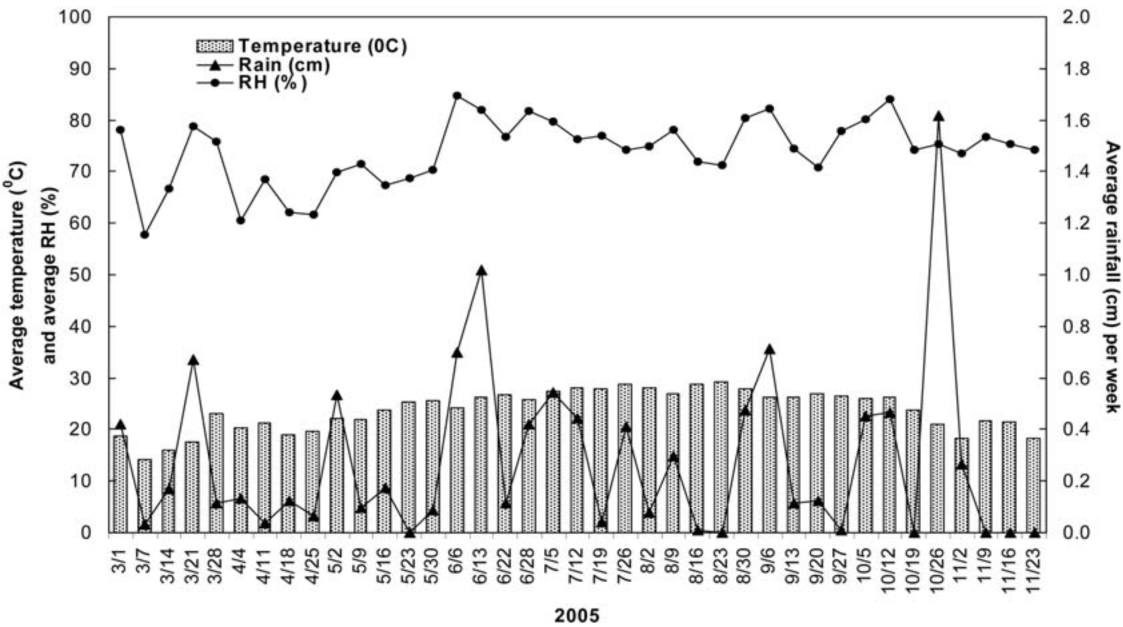

Fig. 2. Average weekly temperature (°C), relative humidity (%), and rainfall (cm) in a Valencia orange grove near Haines City, Florida in 2005.

pesticides applied had no impact on predators, perhaps because oil has a very short residual and imidacloprid is a systemic.

Parasitism by *A. citricola*, as determined by evaluating the pupal chambers of those CLM larvae that survived to the pupal stage, ranged from 0 to 39% over this flush (Fig. 5). There were no differences in parasitism among the 4 treatments ($F = 0.74$, $df = 3$, $P = 0.53$), indicating that oil and imidacloprid did not have a negative effect on *A. citricola* densities in this trial.

Psyllid density categories during the second flush varied by treatment (Table 2). Prior to treatment on 23 Jun, there were no differences in psyllid densities, but after treatment with imidacloprid, a significant reduction in psyllid densities was seen for 3 weeks (28 Jun, 5 and 12 Jul) compared to the untreated control ($F = 25.66$, $df = 3$, $P = 0.001$) (Table 2). By contrast, the growers' oil treatment (treatment B) reduced psyllid densities for only 1 week (7 Jul) compared to the untreated control trees, and the other oil-treated trees (treatment D) did not show a significant difference from the untreated control trees (A). By 19 Jul, there were no differences in psyllid densities among the treatments and again, no differences were observed in densities during the last sample on 26 Jul. No brown citrus aphids were observed in these trees during the second flush.

Third Flush

During the third flush cycle, CLM densities remained relatively low, ranging from 0.05 to 1.27 mines per leaf (Table 1). The number of CLM larvae in the 4 treatments was not different over the entire third flush cycle when densities were combined over the 8 weeks ($F = 0.32$, $df = 3$, $P = 0.80$).

Parasitism by *A. citricola* increased compared to the second flush, ranging from 56% in the untreated control trees to 33% in the oil-treated trees and 22% in the imidacloprid-treated trees (Fig. 5), but these rates were not different among the treatments over this flush cycle ($F = 2.36$, $df = 3$, $P = 0.08$).

Parasitism of the CLM by eulophid parasitoids was not observed during flush cycles 1 or 2, but during flush cycle 3 some pupal chambers contained an unidentified parasitoid. For example, during week 1 of this flush cycle, a total of 7, 34, and 4 pupal chambers were produced in the untreated, imidacloprid- and oil-treated trees, respectively. Of these pupal chambers, 100% of 7 pupal chambers in the untreated control trees, 59% of 34 pupal chambers in the imidacloprid-treated trees, and 25% of 4 pupal chambers in the oil-treated trees contained this unidentified parasitoid; none were found in the growers' oil treatment. During week 2, trees in 2 treatments (untreated and imidacloprid-treated), had 8% (of 12) of the pupal chambers and 67% (of 3) of the pupal chambers, respectively, with an unidentified parasitoid. During weeks 3 and 4, no parasitoids other than *A. citricola* were observed. During week 5, 28% of 18 pupal chambers in the growers' oil treatment contained the unidentified parasitoid. No parasitoids other than *A. citricola* or this

TABLE 1. CITRUS LEAFMINER MINES PER LEAF DURING 3 FLUSH CYCLES IN A VALENCIA ORANGE GROVE NEAR HAINES CITY, FLORIDA IN 2005.

	Mean (± SD) no. of CLM mines per leaf during sample dates							
First flush	1 Mar	7 Mar	14 Mar	21 Mar	28 Mar	4 Apr	11 Apr	
A) Untreated control	0.01 (0.12)	0	0	0	0	0	0	—
B) Grower's oil	0	0	0	0	0	0	0	—
C) Imidacloprid	0	0	0	0	0	0	0	—
D) Oil	0	0	0	0	0	0	0	—
Second flush	22 Jun	28 Jun	7 Jul	12 Jul	19 Jul	26 Jul		
A) Untreated control	0.04 (0.01)	0.13 a (0.08)	0.04 (0.03)	0.05 (0.05)	0.09 (0.02)	0.10 (0.02)	—	—
B) Grower's oil	0.07 (0.03)	0.05 a (0.03)	0.04 (0.03)	0.06 (0.03)	0.09 (0.05)	0.13 (0.08)	—	—
C) Imidacloprid	0.06 (0.04)	0.10 a (0.01)	0.02 (0.03)	0.12 (0.04)	0.06 (0.03)	0.10 (0.02)	—	—
D) Oil	0.04 (0.02)	0.29 b (0.12)	0.13 (0.13)	0.03 (0.03)	0.06 (0.02)	0.15 (0.04)	—	—
P value	0.41	0.001	0.14	0.08	0.45	0.43		
Third flush	5 Oct	12 Oct	19 Oct	26 Oct	2 Nov	9 Nov	16 Nov	23 Nov
A) Untreated control	0.25 (0.25)	1.17 (0.17)	0.55 (0.28)	0.50 (0.30)	0.39 (0.07)	0.32 (0.15)	0.38 (0.17)	0.54 (0.10)
B) Grower's oil	0.05 (0.04)	0.52 (0.49)	0.48 (0.37)	0.33 (0.10)	0.46 (0.14)	0.32 (0.12)	0.36 (0.08)	0.59 (0.30)
C) Imidacloprid	0.29 (0.17)	0.60 (0.22)	0.49 (0.15)	0.33 (0.16)	0.30 (0.06)	0.30 (0.16)	0.40 (0.06)	0.44 (0.11)
D) Oil	1.27 (0.84)	0.65 (0.08)	0.42 (0.16)	0.31 (0.07)	0.28 (0.03)	0.19 (0.10)	0.29 (0.14)	0.44 (0.18)
P value	0.26	0.15	0.93	0.60	0.12	0.71	0.70	0.63

Means were analyzed weekly in each flush cycle by ANOVA and means separated by Fisher's LSD, with $P < 0.05$. Means within a flush cycle within a column with the same letters are not significantly different.

unidentified parasitoid were observed in any samples.

During the third flush cycle, psyllid densities were not different among the 4 treatments during the entire flush cycle when the data were analyzed on a weekly basis (Table 2) or over the entire flush cycle ($F = 0.59$, $df = 3$, $P = 0.62$).

Shoot Length and Shoot Numbers

The number of shoots that could be sampled each week was not different among the treatments during flush cycles 1 ($F = 1.23$, $df = 3$, $P = 0.31$, data not shown) and 2 ($F = 0.77$, $df = 3$, $P = 0.52$). However, during flush cycle 3, there were fewer shoots in the imidacloprid-treated trees over the entire flush cycle ($F = 3.26$, $df = 3$, $P = 0.03$).

The length of each shoot sampled was measured weekly throughout the growing season to document when flush cycles began and ended and to determine whether there were differences in growth rates among the treatments (Fig. 1). During the first flush cycle, there were no differences in shoot lengths among the treatments. During the second flush cycle, there were no significant differences in shoot lengths among the treatments, except that treatment B had significantly longer shoots ($P = 0.03$) on 22 Jun, prior to application of the spray. After that, there were no differences among the treatments, although there was a trend for the trees treated with imidacloprid to have shorter shoots. During flush cycle 3, there were significant differences in shoot lengths on 2 dates when the data were analyzed weekly. On 26 Oct, the imidacloprid-treated trees had shorter shoots (mean = 6.3 cm) compared to the untreated trees (9.6 cm) ($P = 0.008$). Furthermore, when the combined shoot lengths were compared for flush 2 and 3, (post spray), differences were found ($F = 6.29$, $df = 3$, $P = 0.03$), with the imidacloprid-treated shoots significantly

TABLE 2. ASIAN CITRUS PSYLLID NYMPHAL DENSITIES IN A VALENCIA ORANGE GROVE NEAR HAINES CITY, FLORIDA DURING 2005.

	Mean (± SD) score (range 0-4)* each sample date							
First flush	1 Mar	7 Mar	14 Mar	21 Mar	28 Mar	4 Apr	11 Apr	
A) Untreated	0.90 (0.13)	0.98 (0.02)	1.04 (0.04)	1.04 (0.08)	0.89 (0.11)	0.26 (0.13)	0.09 (0.10)	—
B) Grower's oil	0.86 (0.10)	1.00 (0.04)	1.11 (0.07)	1.02 (0.05)	0.79 (0.11)	0.48 (0.08)	0.12 (0.13)	—
C) Imidacloprid	1.01 (0.02)	0.98 (0.02)	1.07 (0.12)	1.01 (0.02)	0.75 (0.11)	0.23 (0.09)	0.11 (0.09)	—
D) Oil	0.96 (0.08)	0.98 (0.05)	0.96 (0.04)	1.02 (0.05)	0.86 (0.09)	0.33 (0.09)	0.14 (0.07)	—
P value	0.20	0.94	0.26	0.94	0.15	0.11	0.95	
Second flush	22 Jun	28 Jun	7 Jul	12 Jul	19 Jul	26 Jul		
A) Untreated	1.08 (0.08)	0.72 a (0.16)	0.82 a (0.10)	0.96 a (0.04)	0.81 (0.17)	0.44 (0.28)	—	—
B) Grower's oil	1.05 (0.01)	0.47 a (0.13)	0.43 b (0.16)	0.97 a (0.05)	0.73 (0.17)	0.38 (0.19)	—	—
C) Imidacloprid	1.28 (0.35)	0.09 b (0.12)	0 c	0.25 b (0.11)	0.68 (0.17)	0.46 (0.17)	—	—
D) Oil	1.17 (0.12)	0.77 a (0.18)	0.78 a (0.21)	1.07 a (0.12)	0.75 (0.05)	0.51 (0.10)	—	—
P value	0.52	0.006	0.0008	0.001	0.79	0.86		
Third flush	5 Oct	12 Oct	19 Oct	26 Oct	2 Nov	9 Nov	16 Nov	23 Nov
A) Untreated	0	0.79 (0.40)	0.92 (0.14)	0.88 (0.12)	0.82 (0.05)	0.96 (0.03)	0.49 (0.15)	0.74 (0.24)
B) Grower's oil	0	0.97 (0.21)	0.73 (0.23)	0.93 (0.07)	0.89 (0.10)	0.91 (0.15)	0.58 (0.23)	0.45 (0.21)
C) Imidacloprid	0.03 (0.05)	0.50 (0.50)	0.88 (0.03)	0.57 (0.25)	0.89 (0.12)	0.77 (0.18)	0.18 (0.17)	0.67 (0.58)
D) Oil	0	0.81 (0.23)	0.68 (0.26)	0.95 (0.05)	0.89 (0.15)	0.97 (0.05)	0.62 (0.29)	0.27 (0.24)
P value	0.45	0.67	0.28	0.08	0.66	0.35	0.09	0.28

*Psyllid densities were scored as: 0 = none, 1 = 1-20, 2 = 21-50, 3 = 51-80 and 4 = >80. Data were analyzed weekly in each flush cycle by ANOVA and means separated by Fisher's LSD, with $P < 0.05$. Means within a flush cycle within a column with the same letters are not significantly different.

shorter. Interpretation of these results is difficult, because there were no differences over the season in CLM densities among the treatments and there were fewer psyllids in the imidacloprid-treated trees during flush cycles 2 and 3 after treatment. The higher psyllid densities in the untreated or oil-treated trees could have caused reductions in growth. Thus, the data suggest that imidacloprid might have detrimental effects on shoot growth and the number of shoots.

Others have found that imidacloprid may have detrimental effects on growth or yield of crops when there are no pest pressures. Obviously, imidacloprid can result in increased crop growth and yield when pest populations exceed the economic injury level and Oosterhuis & Brown (2003) suggested that imidacloprid might promote plant health, stress recovery, and yield increases in cotton. However, McGuire (2005) evaluated imidacloprid for 2 years and failed to find evidence that imidacloprid enhances growth and/or yield in cotton. By contrast, Wu et al. (2004) and Qiu et al. (2004) found that imidacloprid reduced the size of rice grains in treated plants. Hurley & Patel (2003) found that imidacloprid reduced the growth of *Eucalyptus nitens* Deane & Maiden (Maiden) tree seedlings after a root drench at 2 concentrations by 13 and 8%, respectively. Wallace et al. (2000) found that imidacloprid was phytotoxic to cucumbers in the greenhouse and Ebel et al. (2000) found it was toxic to tomatoes and cucumbers in the greenhouse. Dewar et al. (1997) found that

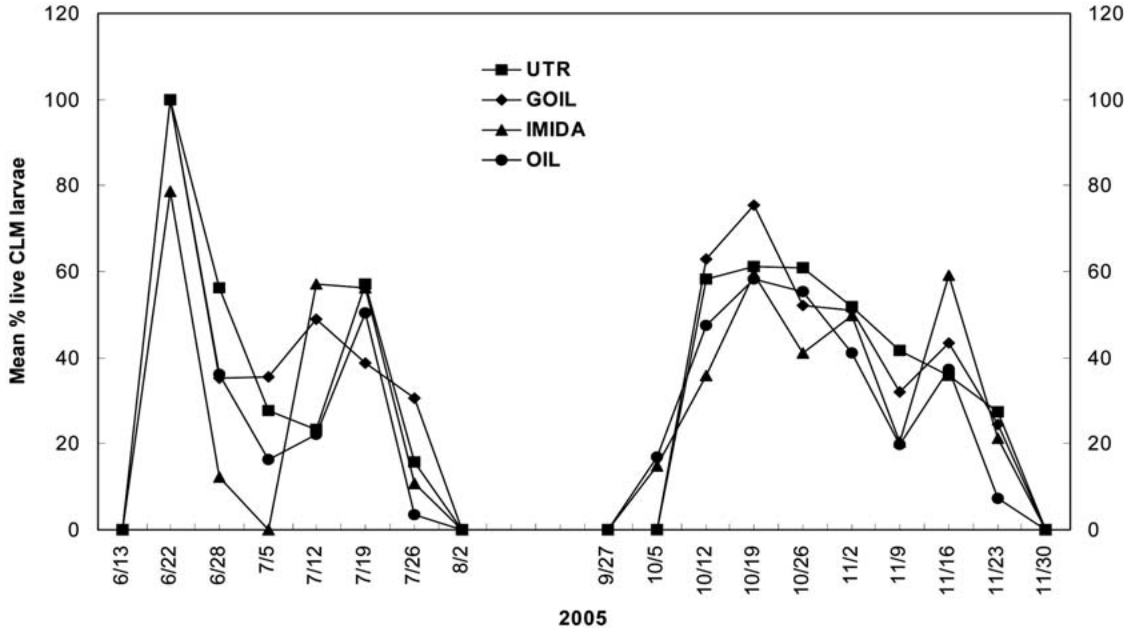

Fig. 3. Mean percentage living citrus leafminer larvae in mines in a Valencia orange grove near Haines City, Florida in 2005.

sugar beet seeds in pellets containing higher rates of imidacloprid had a slower germination rate, and the total number of seedlings emerging was reduced, but different cultivars affected the degree of these effects by imidacloprid. Bhagwat & Lane (2003) found that imidacloprid caused chlorosis of *in vitro* shoot cultures of apples at the end of the 6-week treatment. By contrast, Thielert (2006) reported that imidacloprid protects crops against abiotic stresses such as drought. Our data suggest that imidacloprid could be reducing shoot length in Valencia oranges over the season after a single treatment, but these experiments were not designed to evaluate these effects and there is a possibility that the differences observed are by chance alone. Thus, additional research is needed to confirm any negative effects on growth by imidacloprid in Florida's citrus cultivars. Such research is relevant to developing an IPM program for managing citrus leafminers and Asian citrus psyllids in Florida as a means of reducing the spread of citrus canker and citrus greening disease, respectively, because increased use of imidacloprid to control these disease vectors could have growth or yield costs, as well as benefits.

General Discussion

There were essentially no brown citrus aphids and relatively low densities of psyllid nymphs and CLM larvae in this grove in Polk County, Florida throughout the growing season during 2005. The density of psyllids was estimated by an abundance score, with only nymphs being estimated, because previous experience in monitoring psyllid populations in a grapefruit grove in the Ft. Pierce area during 2004 had found that high rates of predation occurred on eggs and newly hatched nymphs (Hoy et al., unpubl.).

Prior to this study, no information was available on the phenology of *A. citricola* and CLM in this citrus-growing area ('the Ridge') of Florida. These results indicate that *A. citricola* is an important natural enemy of the CLM, as shown by the proportion of those CLM larvae that survived to the pupal stage during 2005 in both treated and control trees. As expected, *A. citricola* populations lagged behind their CLM host during flush cycle 2.

A large number of empty mines were observed in all 4 treatments. Empty mines are often due to predation by ants (Amalin et al. 2002; Zappala et al., 2007). Some dead larvae appeared to have been fed on by lacewing larvae or spiders. Previous work by Browning & Peña (1995) and Amalin et al. (1996, 2002) found that green lacewing larvae (*Chrysoperla rufilabris* (Burmeister)), ants (especially the red imported fire ant, *Solenopsis invicta* Buren), thrips, hunting spiders (*Chiracanthium inclusum* (Hentz), *Hibana velox* (Becker) and *Trachelas volutes* (Gertsch)), and mirid bugs are predators of CLM larvae in lime orchards in south Florida, causing approximately 34 to 39% of the mortality observed. Villanueva-Jimenez et al. (2000) found that total morality of the CLM in a Gainesville, FL grapefruit grove var-

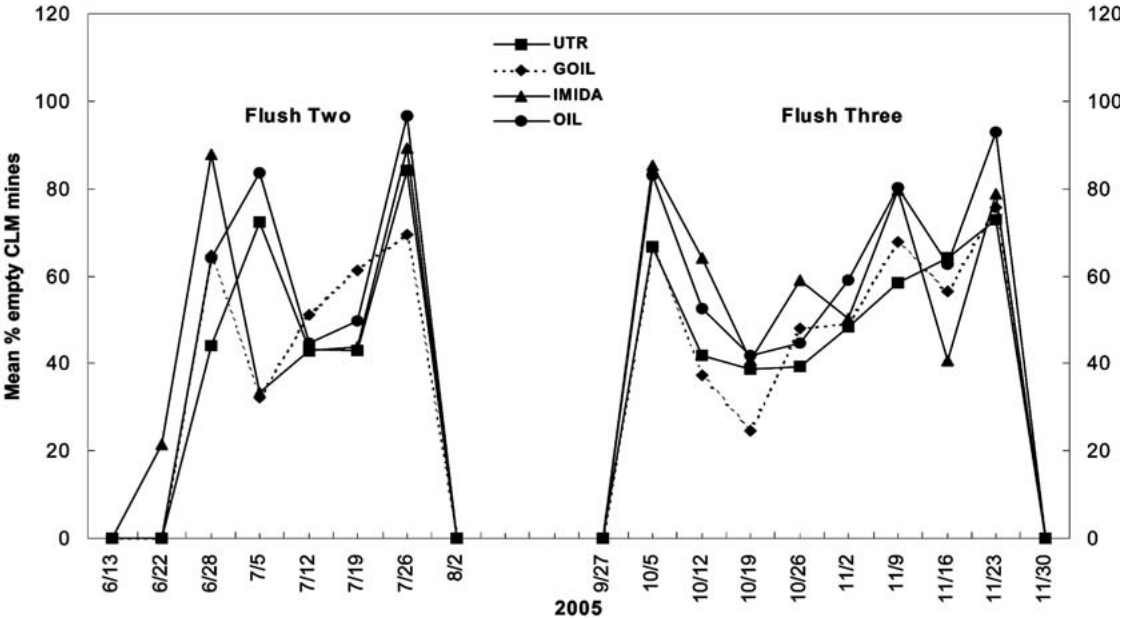

Fig. 4. Mean percentage empty citrus leafminer mines in a Valencia orange grove near Haines City, Florida in 2005.

ied throughout the season, but was greater than 70% after the first flush cycle, with "unexplained larval mortality" that was as high as 70.4% during the fourth flush during 1997. In that study, 32 to 80% of the mortality was caused by *A. citricola* on those CLM that managed to reach the pupal stage (Villanueva-Jimenez et al. 2000), but empty mines also were observed and could have been due to predation by red imported fire ants, which were abundant in the grove. Zappalá et al. (2007) found that red imported fire ants removed CLM larvae that had been parasitized by *A. citricola* from mines in laboratory and field trials.

From about Jul through Nov in this Valencia orange grove in central Florida, a substantial proportion of the few CLM larvae that survived to the pupal stage were parasitized by *A. citricola*; this mortality factor would help to reduce the number of adults entering the winter. However, the proportion of CLM pupae that were parasitized by *A. citricola* was lower than expected, for unknown reasons. Previous samples during Aug through Oct in multiple sites in Florida had found up to 99% of the pupae parasitized by *A. citricola* (Hoy et al. 1995; Hoy & Nguyen 1997; Pomerinke & Stansly 1998; Villanueva-Jimenez et al. 2000). The reason(s) for the relatively lower parasitism rates by *A. citricola* in this citrus grove near Haines City is unknown.

We are unable to conclude that the combined action of natural enemies and pesticide applications suppressed CLM densities below the economic threshold, because it is not known what an economic injury level is in Valencia oranges grown in the 'Ridge' area of Florida, especially now that the canker eradication program has ended (in 2006) and canker is considered established, although there was no canker in the grove at the time of this study. When the CLM attacks trees in nurseries and young trees in groves, direct damage by the CLM can delay growth and alter canopies as well as open the mines to infection by the canker bacterium. The economic impact of CLM on mature orchards in areas where citrus canker is now endemic in Florida is not yet known, and depends on canker bacterial density, weather conditions, tree age, and timing of the damage. It is unclear if there is a surplus of leaf area in citrus grown in central Florida, although Knapp et al. (1995) suggested that a 10% leaf area loss due to CLM mines did not affect yield (prior to canker establishment). In Florida, CLM densities typically are very low during winter and during the first spring flush, but increase during the growing season to peak in the fall, and this pattern was followed in the Valencia grove studied. In China, Huang & Li (1989) found that leaf area loss of less than 20% did not affect yield, and suggested that a loss of 15% of leaf area, or about 0.74 CLM larvae per leaf, was the economic threshold. Garcia-Marí et al. (2002) evaluated the economic injury level of CLM in the Valencia area of eastern Spain from 1996 to 1999 and found that 5-15% of the annual new leaf area of mature trees could be damaged without affecting yield, primarily because the production of new shoots was concentrated

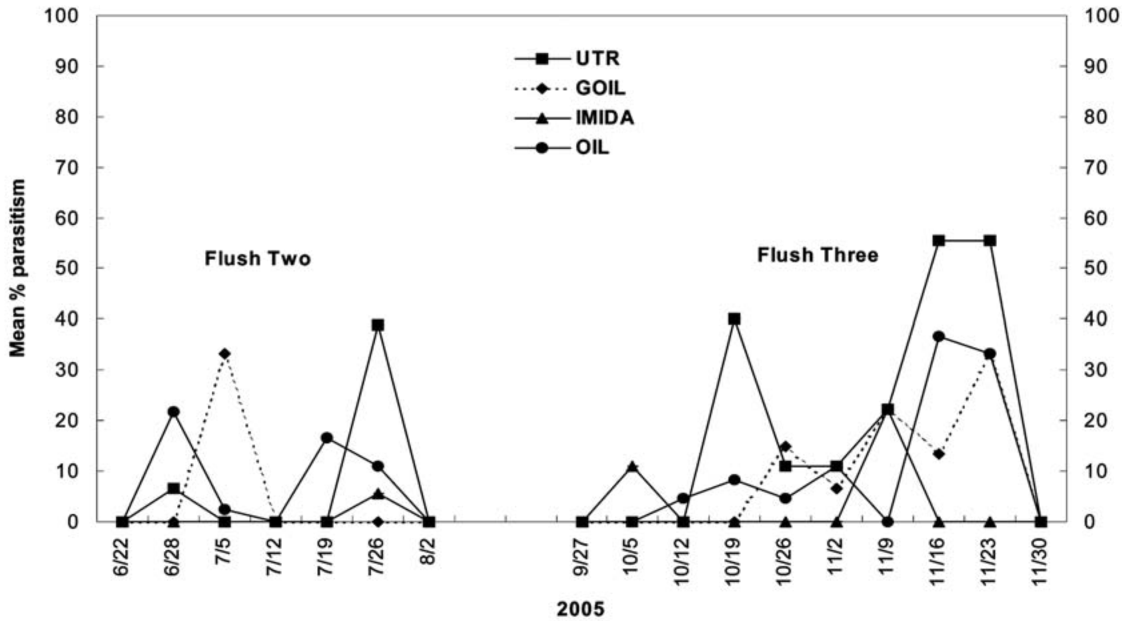

Fig. 5. Mean percentage citrus leafminer pupae parasitized by *Ageniaspis citricola* near Haines City, Florida in 2005.

early in the spring when CLM densities were very low but high CLM populations occurred in summer and fall so the citrus leafminer's effect on biomass, yield and fruit quality was minimal. If this damage level is used to assess potential growth or yield loss for the Valencia grove in this study, then none of the trees reached this level of infestation during either the first or second flush. During the second flush, the maximum density of CLM mines/leaf averaged 0.29 (SD ± 0.12) in the trees treated with oil, while the maximum number of CLM mines/leaf was 0.13 (0.08) in the untreated control. During the third flush, CLM densities peaked in the untreated control and in one set of oil-treated trees at 1.17 (0.22) and 1.27 (0.80) mines/leaf, respectively, but these densities were found only during 1 week. Thus, there is no evidence that the treatments (oil or imidacloprid) significantly reduced CLM densities. The imidacloprid treatment did reduce psyllid densities for 3 weeks, but may have reduced shoot length. The possibility of a detrimental effect by imidacloprid on shoot growth and shoot number should be investigated, particularly if multiple applications of imidacloprid are applied to suppress psyllid populations in an effort to reduce transmission of greening disease in Florida.

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