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FORTUITOUS ESTABLISHMENT OF *AGENIASPIS CITRICOLA* (HYMENOPTERA: ENCYRTIDAE) IN JAMAICA ON THE CITRUS LEAFMINER (LEPIDOPTERA: GRACILLARIIDAE)

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The citrus leafminer *Phyllocnistis citrella* Stainton (CLM) is endemic to India, China and other Asian countries (Clausen 1931), but has spread rapidly into other citrus-growing areas throughout the world during the past 15 years. The CLM was first noticed in Florida in 1993 (Heppner 1993) and spread throughout the Caribbean, as well as Central and South America. A classical biological control project was initiated and the host-specific endoparasitoid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) was imported and released in Florida (Hoy & Nguyen 1994, 1997). The Australian population of *A. citricola* had been imported from Thailand into Australia (Neale et al. 1995) prior to being released into Florida. Subsequently, another *A. citricola* population from Taiwan was imported and introduced into Florida (Lo & Chiu 1988; Hoy et al. 2000). Individuals from Taiwan and Thailand appear morphologically identical, but slight differences in biology and behavior were noted (Ujiye et al. 1996; Yoder & Hoy 1998), which led to molecular studies to characterize the two populations (Hoy et al. 2000; Alvarez & Hoy 2002). Hoy et al. (2000) showed, with RAPD-PCR and DNA sequence analysis of *Actin1* and *Actin2* genes, that there are likely 2 cryptic species of *Ageniaspis* parasitizing the CLM, 1 from Taiwan and 1 from Thailand. Both populations were released in Florida, and current, but limited, evidence suggests that only the population originating from Thailand established (Alvarez 2000).

While monitoring populations of the CLM in Jamaican citrus groves between 24 and 29 May 2004, high rates of parasitism (ranging from 30-90%) of the CLM by *Ageniaspis* were found throughout the island. To further evaluate the distribution and abundance of *A. citricola* in Jamaica, a survey was conducted between Jan and Sep 2005 at 4 sites in the citrus-growing regions of Jamaica. Tender new growth on citrus trees was monitored at these sites to determine the number of leafminer mines per leaf and to determine the proportion of pupal chambers that contained pupae of the citrus leafminer or *A. citricola*. A mean (±S.D.) of 41.3 (26.9)% of all pupal chambers examined contained *A. citricola* in these sites sampled during Jan-Sep 2005. On most sample dates when CLM was found, fewer than the targeted number of 40 pupal chambers could be sampled because leafminer densities were very low, averaging 0.50 (0.25) mines per leaf (Table 1). These survey data suggest that CLM populations are low in Jamaica and the establishment and abundance of *A. citricola* appears to be contributing to this status.

No purposeful releases of *A. citricola* were made in Jamaica and no one knows how this par-

TABLE 1. SURVEY OF JAMAICAN CITRUS-GROWING AREAS FOR INCIDENCE OF PARASITISM OF THE CITRUS LEAFMINER BY *AGENIASPIS CITRICOLA* DURING 2005.

Parish	Location	Sample Date	Total no. flush/total no. leaves sampled	Mean mines/ leaf (SD)	No. pupal chambers examined*	% chambers with *A. citricola*
Clarendon	Four Paths	27 September	6/52	0.65 (0.22)	17	6%
St. Catherine	Bog Walk	27 January	19/214	0.28 (0.17)	26	27%
		28 February	20/297	0.31 (0.18)	40	35%
St. Elizabeth	Balaclava	3 February	20/255	0.22 (0.10)	14	64%
		9 June	20/396	0.99 (0.29)	40	23%
St. Mary	Highgate	21 April	20/182	0.49 (0.25)	25	40%
		5 May	16/157	0.59 (0.26)	14	93%
		31 May	20/180	0.46 (0.22)	40	43%

*All pupal chambers up to 40 per sample were examined. On some dates, fewer than 40 chambers were examined because only CLM larvae were present in the mines at that site.

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asitoid managed to colonize the island. However, because Jamaican growers had never found CLM populations to cause serious damage to citrus, except for occasional population increases in nurseries and newly established trees (personal communication, Dr. Percy Miller, Citrus Growers Association, 2006), we speculate that *A. citricola* entered Jamaica with the citrus leafminer on citrus, and multiplied and spread throughout Jamaica. This conclusion was reached because the CLM is unable to develop on host plants other than citrus and that *A. citricola* is host specific to the CLM.

Adult parasitoids collected in 95% ethyl alcohol (EtOH) during May 2004 were taken to Gainesville, FL where they were analyzed to determine whether they were derived from the Taiwan or the Thailand populations of Ageniaspis by using *Actin* gene sequence data (Hoy et al. 2000). Genomic DNA from individual wasps was extracted by the PureGene method according to the manufacturer's recommendations (Gentra, Minneapolis, MN) and resuspended in 10-µL of sterile water. High-fidelity PCR amplifications were performed by resuspending 1 µL of DNA in a 50-µL reaction containing 400 picomoles of degenerate primers (forward: MHO105, 20-mer, 5'TGGGAYGAYATGGARATHTGGCAYCA-3'; reverse: MHO99, 20-mer, 5'GCCATYTCYTGYTCRAARTC-3', where Y = C or T, R = A or G, and H = A or C or T). The primers were expected to amplify a 400-bp region, 266 to 666 bp from the start site of the *Actin* gene family, which typically contains 6 different genes in insects. The primers were added to 50 mM Tris pH 9.2, 1.75 mM MgCl2, 16mM ammonium sulfate, 350 µM dATP, dGTP, dCTP, dTTP and 0.2 units of *Tgo* and 1.0 units of *Taq* DNA polymerase (Roche Biochemicals, Indianapolis, IN). High-fidelity PCR amplification was performed for 35 cycles, with 3 linked profiles: (1) one cycle consisting of 94°C denaturation for 2 min; (2) 10 cycles consisting of 94°C denaturation for 10 s, 49°C annealing for 30 s and 68°C extension for 1 min; and (3) 25 cycles consisting of 94°C denaturation for 10 s, 49°C annealing for 30 s and 68°C extension for 1 min plus 20 s added for every consecutive cycle.

The 400-bp *Actin1* gene sequences from the Australian and Taiwan populations show 20 base-pair differences between them (95% similarity), while the 400-bp *Actin2* gene sequences show 12 base-pair differences (97% similarity) (Hoy et al. 2000). These *Actin* sequence differences are similar to the degree of sequence divergence found for other highly conserved genes and are equivalent to the differences found in *Actin* sequences from different genera. Hence, the *Actin* sequence differences observed between the Australian and Taiwan parasitoids indicate that they belong to different cryptic species.

Twelve clones were sequenced from the Jamaican *Ageniaspis* and 9 of the *Actin* sequences were identical to *Actin1*. Two clones produced sequences that were identical to *Actin2* sequences from the Australian population of *A. citricola*, which was collected originally from Thailand. In addition, a third, and new, *Actin* sequence was found, with an 85% similarity to the *Actin1* sequence of the Australian population. Because insects have 6 *Actin* genes, this new sequence will be called *Actin3* and will be deposited in GenBank (Accession Number EF149011).

Summary

These data indicate that the population of *A. citricola* in Jamaica probably originated from the Australian (Thailand), rather than from the Taiwan, population. This is consistent with what is currently known about the origin of the established *Ageniaspis* population in Florida (Alvarez 2000). It is not known when, or how, *A. citricola* arrived in Jamaica, although the CLM was detected there in 1994. The fortuitous establishment of *A. citricola* on the CLM in Jamaica is not the only such establishment of a natural enemy discovered during this 2004 survey of citrus. The parasitoid *Lipolexis oregmae* Gahan (Hymenoptera: Aphidiidae) was found attacking the brown citrus aphid, *Toxoptera citricida* Kirkaldy (Hemiptera: Aphididae) (Hoy et al. unpublished data), and the eulophid parasitoid *Tamarixia radiata* Waterston was found attacking the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). The fact that 3 natural enemies of 3 invasive citrus pests were found in Jamaica, none of which were purposefully imported and released, suggests that pest-infested citrus trees were imported into Jamaica without going through appropriate quarantine procedures. Because each pest arrived at different times, the parasitoids probably arrived at different times, as well. This indicates that an analysis is needed to identify the critical control points within those services in Jamaica that support border protection, and that procedures may require strengthening.

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