Proc. Fla. State Hort. Soc. 116:224-226. 2003.

BIOLOGICAL CONTROL OF THE CITRUS LEAFMINER WITH *AGENIASPIS CITRICOLA* (HYMENOPTERA: ENCYRTIDAE) IN LOUISIANA

SETH J. JOHNSON[1] AND DONALD C. HENNE
LSU Agricultural Center
Department of Entomology
402 Life Sciences Building
Baton Rouge, LA 70803

WAYNE J. BOURGEOIS
LSU Agricultural Center
Citrus Research Station
22193 Highway 23
Port Sulphur, LA 70083

Additional index words. Phyllocnistis citrella, parasitoids

Abstract. **The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was discovered in Louisiana in May 1994. A classical biological control program was initiated against the citrus leafminer in Louisiana during January 1995. The parasitic wasp, *Ageniaspis citricola* Logviniskaya, was obtained from the University of Florida, and was reared at the LSU Agricultural Center, Citrus Research Station in Plaquemines Parish, La. This parasitoid was released there and at three other locations in the parish during June and July 1995. Mean parasitism by *A. citricola* at survey sites peaked at 37%, 37% and 56% in 1995, 1996 and 1997, respectively. *Ageniaspis citricola* appeared to have established but disappeared in 2000, apparently due to the severe drought of 1999 and 2000. Several hundred *A. citricola* pupae were collected in Gainesville, Fla. on 8 July 2002. A total of 130 *A. citricola* adult wasps were released at the Citrus Research Station on infested *Citrus unshi* Marcovitch 'Satsuma" Mandarin orange trees from 12-15 July 2002. The first *A. citricola* was recovered on 8 August. Mean parasitism by *A. citricola* peaked at 51% on 18 September 2002.**

The citrus leafminer, a native of India and Southeast Asia, is a serious invasive pest of citrus throughout the world (Hoy and Nguyen, 1997). Within a three-year span from 1993 to 1996, it underwent a rapid rate of dispersal, invading the citrus growing-countries of the Mediterranean, the Caribbean, South America and North America (Hoy and Nguyen, 1997). The citrus leafminer was first found in Louisiana in May 1994 (Johnson et al., 1998). The larva feeds on epidermal cells, producing broad serpentine mines in the leaves and can cause serious damage if populations are unchecked. High densities of leafminers cause twisted and damaged leaves that dry out, have reduced rates of photosynthesis and, under severe conditions, may defoliate.

Management of the citrus leafminer has focused primarily on biological control because there are problems with the other methods of control. Chemical control of citrus leafminer over the long term is an inappropriate strategy due to high costs, potential development of resistance, disruption of biological control agents of other citrus pests, concerns about pesticide residues, and effects on non-target organisms. Citrus leafminer resistance to pyrethroids and organophosphate insecticides has been documented in China (Tan and Huang, 1996). In China cultural control is the traditional management method for citrus leafminer and includes sanitation, pruning, bud removal, proper fertilization and monitoring. Trees are allowed to bud only during late fall, which allows growth to occur when pest populations are lowest (Tan and Huang, 1996). Unfortunately, it is impossible to achieve this type of control in Louisiana because summer rainfall is generally abundant, favoring a frequent tree flushing pattern when leafminer populations are highest. Host plant resistance is not feasible over the short term because field tests have confirmed widespread susceptibility of citrus species and rootstocks to the citrus leafminer (Jacas et al., 1997).

Classical biological control projects using *A. citricola* for control of citrus leafminer have been initiated in many countries (Hoy and Nguyen, 1997). *Ageniaspis citricola* is an arrhenotokous, gregarious and polyembryonic endoparasitic encyrtid wasp and produces 1-10 progeny per leafminer host (Edwards and Hoy, 1998). Female *A. citricola* oviposit in the egg and first instar citrus leafminer. Parasitoid development is not completed until the prepupa has formed its pupal chamber on the leaf edge (Edwards and Hoy, 1998). In the United States, *A. citricola* was released in Florida in 1994 (Hoy et al., 1995), and in Texas in 1995 (Legaspi et al., 1999). In Florida, *A. citricola* is well established, widely distributed and abundant and is the dominant natural enemy of citrus leafminer (Hoy and Nguyen, 1997), but the parasitoid did not establish in south Texas (Legaspi et al., 1999). We report here the successful release and establishment of *A. citricola* in Louisiana in the mid-1990s, its disappearance in 1999-2000 and efforts to reestablish it in 2002.

Materials and Methods

A classical biological control project against the citrus leafminer with *A. citricola* was initiated in January 1995. The parasitoids were obtained from a colony at the University of Florida at Gainesville. A colony was established at the LSU Agricultural Center, Citrus Research Station, Port Sulphur, Louisiana, using rearing procedures that were a modification of the approach of Smith and Hoy (1995). Synchronized rearing of the three principal organisms: citrus leafminer, *Citrus sinensis* 'Navel' orange trees and *A. citricola* was conducted in one greenhouse (29.3 × 9.1 m). Three screened (32 × 32 mesh polyethylene cloth) cages (3.7 × 2.7 × 2.3 m) within the greenhouse were used to maintain a pure colony of *A. citricola* and concentrate citrus leafminer egg and first instar larval density prior to exposure to *A. citricola*. Greenhouse temperature was maintained at 27.5 ± 2 °C until April, when the sides were raised to reduce overheating. Overhead mist sprinklers were operated for 15 min at 8:00 am and 1:00 pm to maintain high humidity in the cages. Groups of 40 trees (1-2 year old and 0.6-1.2 m in height) were flushed by pruning and fertilization at two-week intervals to maintain suitable host plant material for citrus leafminer oviposition. Between March and June, we received several shipments of *A. citricola* and were able to cycle

[1]Corresponding author.

it for two generations between March and April. On 18 June, 80 adult *A. citricola* were released into two of the screen cages with 20-30 citrus trees each infested with two to three leafminers per leaf. On 21 June, the trees were moved from the cages into the open-sided greenhouse. *Ageniaspis citricola* that emerged dispersed from the greenhouse to the adjacent citrus grove. We also made a small release of six to 25 adult *A. citricola* at three locations (27 km N, 13 km S and 47 km S of the Citrus Research Station) on 17 July.

A survey was conducted in Plaquemines Parish, La. between 1995 and 1997 and in 2002 to measure percent parasitism by *A. citricola* and native parasitoids and to determine *A. citricola*'s dispersal rate. Ten sites were sampled in 1995 and 1996 and six sites in 1997 and 2002. The sample unit was a terminal containing 8-12 leaves with the apical leaves partially expanded and 12 to 25 mm in length. One terminal was randomly collected from the middle region of 10 trees at each site. Both Navel and Satsuma orange trees were sampled, because neither the citrus leafminer nor *A. citricola* has a known preference. Leaf samples were placed in plastic bags and transported in a cooler to the laboratory. Each sample was examined under a dissecting microscope and citrus leafminer larvae and pupae counted and sorted. Larvae and pupae were held in 100 × 15 mm Petri dishes lined with moistened filter paper and held on a counter top at 25 °C. Samples were examined at four-day intervals under a dissecting scope for either parasitoid or adult leafminer emergence. In 1997, approximately 100 citrus leafminer pupal cells were collected at each site and percent parasitism by *A. citricola* was measured. Parasitoids were pinned on points and voucher specimens deposited in the Louisiana State University Agricultural Center Entomology Museum. Parasitoids were identified by Dr. Michael Schauff with the Systematic Entomology Laboratory, U.S. Dept. Agriculture, Museum of Natural History Washington, D.C.

In 2002, a decision was made to release *A. citricola* in Louisiana again after failing to locate it in 2000 and 2001. Several hundred pupae of *A. citricola* were collected from citrus foliage infested with citrus leafminer in a greenhouse and shade house at the Entomology Department of the Univ. of Florida in Gainesville, Fla. on 8 July 2002. The pupae were transported in 50 mL plastic tubes in a cooler to the Entomology Department of the LSU Agricultural Center in Baton Rouge, La. The pupae were held at 27 °C in plastic bags with citrus foliage to provide high humidity until wasp emergence. A total of 130 adult *A. citricola* emerged and were released at the Citrus Research Station from 12-15 July on Satsuma trees infested with citrus leafminer eggs and first-instar larvae.

Results and Discussion

The first recovery of *A. citricola* at the Citrus Research Station was on 12 July 1995. The first survey date on 9 August 1995 found *A. citricola* distributed 42 km north and 26 km south of the Citrus Research Station. The percentage of leaves infested with citrus leafminer peaked at 52.7 on 9 Aug. and declined over the survey to 25.5 on 27 Sept. The citrus leafminer larval density was 0.75 per leaf on 9 Aug. and declined to 0.3 per leaf on 27 Sept. Parasitism by *A. citricola* at the sites ranged from zero to 100% and peaked at an average of 37% on 27 September. Several native parasitoids were identified from the survey sites including *Elasmus tischeriae* (Howard), *Pnigalio* sp., and *Zagrammosoma multilineatum* (Ashmead). However, their combined parasitism level was much lower than *A. citricola* and peaked at an average of 7% on 27 Sept.

In 1996, the citrus leafminer was found in late June and *A. citricola* was not found until 6 Aug. Late freezes in Mar. 1996 and a dry spring delayed flush by citrus and also the citrus leafminer population. The average percentage of leaves infested with citrus leafminer was 52.9 on 9 Sept., the last sample date of 1996. The citrus leafminer larval density peaked at 0.88 per leaf on 9 Sept. Parasitism by *A. citricola* at the sites ranged from zero to 96%, and peaked at an average of 37% on 9 September. Mean parasitism by the three native parasitoids peaked at 2% in 1996.

In 1997, the first *A. citricola* was found on 29 July. Parasitism by *A. citricola* at the survey sites ranged from zero to 100% and peaked at an average of 56% on 12 Sept. Parasitism by the three native parasitoids peaked at an average of 8% on 12 Sept.

The parasitoid *A. citricola* disappeared in 2000. No *A. citricola* were found in samples of 400-500 citrus leafminer pupae examined at two- to three-week intervals from July to September in 2000 and 2001. During this period, the citrus leafminer population exploded and most of the mid- and late-summer flush was infested with multiple mines in untreated orchards. In 2002, the citrus leafminer population was high and peaked at an average of 3.0 larvae per leaf on 8 Aug. (Fig. 1). Damage was also severe, with an average of 54.5 to 96.4% of leaves infested with larval mines between 27 June and 18 Sept. *Ageniaspis citricola* was recovered on 8 Aug., less than a month after the re-release, and parasitism peaked at an average of 51% on 18 Sept. (Fig. 1).

Population levels of the citrus leafminer before and after the disappearance of *A. citricola* clearly demonstrate the importance of this parasitoid to citrus leafminer control in Louisiana. Prior to 2000, the citrus leafminer population remained below an average of one larva per leaf but after 2000, and the disappearance of *A. citricola*, the citrus leafminer population exploded to an average of 3.0 larvae per leaf in 2002. The few native parasitoid species attacking the citrus leafminer in Louisiana, which provided a maximum of 8% larval mortality between 1995 and 1997, did not replace the mortality provided by *A. citricola* after it disappeared. General

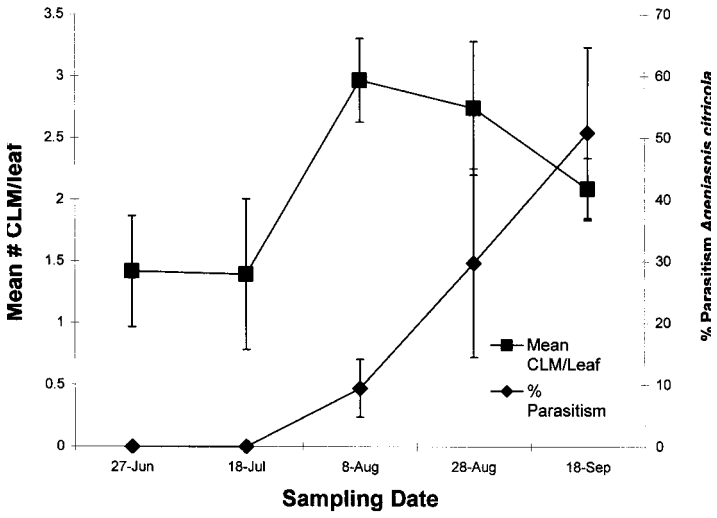

Fig. 1. Citrus leafminer (CLM) density and % parasitism by *Ageniaspis citricola* in Plaquemines Parish, Louisiana in 2002. Parasites (130) released at Citrus Research Station, Port Sulphur, LA between 12 and 15 July.

predators also provide larval mortality and several have been identified in Florida (Amalin et al., 2001) but they, like the native nonspecific parasitoids, did not replace the mortality caused by the specific parasitoid, *A. citricola*. Clearly, in Louisiana, the mortality caused by *A. citricola* was irreplaceable mortality. If *A. citricola* becomes reestablished and causes pre-2000 levels of mortality and the citrus leafminer's populations return to levels of below one larva per leaf, it will provide strong evidence of the important regulatory role of *A. citricola*.

Acknowledgments

We thank Dr. Marjorie A. Hoy, Department of Entomology and Nematology, University of Florida, for providing *A. citricola* in 1995 and assistance in collecting the parasitoid in Gainesville in July 2002. Thanks are extended to Drs. Gregg Henderson and Abner Hammond, LSU Agricultural Center, for their helpful comments and criticisms of an earlier draft of this manuscript. Approved for publication by the Director, Louisiana Agricultural Experiment Station as Manuscript No. 03-26-1339.

Literature Cited

Amalin, D. M., J. E. Pena, R. E. Duncan, H. W. Browning, and R. McSorley. 2001. Natural mortality factors acting on citrus leafminer, *Phyllocnistis citrella*, in lime orchards in south Florida. Biocontrol 47:327-347.

Edwards, O. R. and M. A. Hoy. 1998. Biology of *Ageniaspis citricola* (Hymenoptera: Encyrtidae), a parasitoid of the leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Ann. Entomol. Soc. Am. 91:654-660.

Hoy, M. A. and R. Nguyen. 1997. Classical biological control of the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae): Theory, practice art and science. Trop. Lepidop. 8(Suppl. 1):1-19.

Hoy, M. A., R. Nguyen, D. Hall, R. Bullock, M. Pomerinke, J. Pena, H. Browning, and P. Stansly. 1995. Establishment of citrus leafminer parasitoid, *Ageniaspis citricola* in Florida. Citrus Industry 76:12-17.

Jacas, J. A., A. Garrido, C. Margaix, J. Forner, A. Alcaide, and J. A. Pena. 1997. Screening of different citrus rootstocks and citrus-related species for resistance to *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Crop Protection 16:701-705.

Johnson, S. J., A. Vaughn, and W. J. Bourgeois. 1998. Biological control of citrus leafminer in Louisiana. Louisiana Agric. 41:11-14.

Legaspi, J. A., J. V. French, M. E. Schauff, and J. B. Wooley. 1999. The citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in south Texas: Incidence and parasitism. Florida Entomol. 82:305-316.

Smith, J. M. and M. A. Hoy. 1995. Rearing methods for *Ageniaspis citricola* (Hymenoptera: Eulpohidae) released in a classical biological control program for the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Florida Entomol. 78:600-608.

Tan, B. and M. Huang. 1996. Managing the citrus leafminer in China, p. 49-52. In M. A. Hoy (ed.). Managing the citrus leafminer. Proc. Intern. Conf., Orlando, Florida, April 23-25, 1996. Univ. of Fla., Gainesville. 119 pp.