ESTABLISHMENT OF *AGENIASPIS CITRICOLA* (HYMENOPTERA: ENCYRTIDAE) FOR BIOLOGICAL CONTROL OF *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE) IN FLORIDA

MARK A. POMERINKE AND PHILIP A. STANSLY
Southwest Florida Research and Education Center
Institute of Food and Agricultural Science, University of Florida
P.O. Drawer 5127 Immokalee, FL 34143-5002

ABSTRACT

The parasitoid *Ageniaspis citricola* (Hymenoptera: Encyrtidae), originally from Thailand and obtained from Australia, was released at 52 sites in Southwest Florida between May 1994 and September 1995 as part of a statewide program of biological control of citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Establishment and over-wintering was confirmed during spring of 1995 and 1996 in spite of frosts experienced during the previous winters. Parasitism from *A. citricola* at monitored groves increased from 2% in May 1994 to 86% in October 1995, apparently unhindered by native parasitoids. In contrast, apparent parasitism of citrus leafminer from endemic parasitoids fell from 30% to 2% during the same period. Wind-aided dispersal of *A. citricola* occurred in all directions and was documented to a maximum of 48 km from the nearest release point. *A. citricola* is now ubiquitous throughout the region and will probably remain a permanent component of the entomophagous complex using citrus leafminer.

Key Words: citrus leafminer, *Ageniaspis citricola*, biological control, *Phyllocnistis citrella*

RESUMEN

El parasitoide *Ageniaspis citricola* (Hymenoptera: Encyrtidae), que originalmente es de Tailandia pero qué fue obtenido para este proyecto en Australia, fue liberado en 52 sitios de la zona citrícola del sudoeste de Florida entre Mayo de 1994 y Septiembre de 1995 como parte de un programa estatal del control biológico del minador de los cítricos, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Durante las primaveras de 1995 y 1996 se confirmó su establecimiento a pesar de algunas heladas durante los inviernos anteriores. Parasitismo por *A. citricola* en plantaciones monitoreadas aumentó del 2% en Mayo de 1994 al 86% en Octubre de 1995, aparentemente sin impedimento por parasitoides nativos. En cambio, parasitismo del minador por parasitoides nativos bajó del 30% al 2% durante el mismo período. Se documentó la dispersión de *A. citricola*, aparentemente por medio del viento, en todas direcciones, con el máximo de 48 km de distancia del sitio de liberación más cercano. Ya se encuentra *A. citricola* en toda la región y se espera que sea un componente permanente del complejo entomófago que utiliza a *P. citrella*.

Citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was detected in May, 1993 infesting Persian limes in Dade County, Florida (Heppner 1993). The moth spread quickly across Florida, and within several months was found in all citrus growing regions of the State (Knapp et al. 1993). It has since been de-

tected throughout the Caribbean, Central America (Heppner 1995), South America, and most Mediterranean countries (Knapp et al. 1993, Hoy and Nguyen 1997). Citrus leafminer had previously been reported as a major pest of new flush in southeast Asia, Australia, Japan, Taiwan, and South Africa (Heppner 1995). Larvae feed by mining the epidermal layer of leaves (Sonhi and Verma 1965), stems, and occasionally fruit.

Evidence from the United States, India, Japan, and Israel indicate that citrus leafminer can be managed by biological control from native and introduced parasitoids (Peña et al. 1996, Batra and Sandhu 1981, Ishii 1953, Argov and Rossler 1996). Surveys in 1993 and 1994 revealed that the leafminer was attacked by a complex of native parasitoids in the families Eulophidae, Encyrtidae, Elasmidae, Eurytomidae and Pteromalidae (LaSalle and Schauff 1996). Peña et al. (1996) found that generalist native parasitoids provided low to moderate levels of *P. citrella* parasitism in lime orchards in southeast Florida. They suggest that this reduction in effectiveness of native parasitoids may be the result of pesticide applications for the control of citrus pests. Given the apparent low levels of control by native parasitoids and difficulty in achieving good chemical control due to the biology of citrus leafminer (Knapp et al. 1995) the importation and release of additional biological control agents was deemed necessary.

Citrus leafminer had been detected in Australia in 1912 (Beattie 1993), and the gregarious parasitoid *Ageniaspis citricola* Logvinovskaya from Southeast Asia was introduced there in 1989 (Neale et al. 1995). *Ageniaspis* was imported from Australia to Florida with permission from that government in spring 1994. Federal and Florida state permits to release *A. citricola* from quarantine were granted for adults field-collected as pupae in May of that year (Hoy & Nguyen 1994). *A. citricola* is an internal koinobiont parasitoid of Citrus leafminer. Eggs are laid into eggs or first instar leafminer and parasitoids pupate inside the host prepupa, located within its pupal cell (Pomerinke, personal observation). The only reported host of *A. citricola* is Citrus leafminer (Hoy 1994), although development in the Mahogany leafminer, *Phyllocnistis erechtiisella* Chambers, has been observed (Stansly, unpublished data).

The following study was conducted to document establishment and dispersal of A. citricola, and its effects on incidence of native parasitoids. We report results of 13 releases at 10 groves (a total of 2,625 *A. citricola* adults) in 1994, and 39 releases at 25 groves (a total of 9,757 *A. citricola* adults) in 1995 made in five counties in Southwest Florida: Henry, Lee, Collier, Charlotte, and Glades.

Materials and Methods

Parasitoids were received from Gainesville, Florida, rearing facilities in plastic 50 ml vials containing an average of 100 adults. Wasps were provided with strips of filter paper moistened with pure honey and packed in Styrofoam boxes ($21 \times 21 \times 31$ cm) cooled with EverCold® gel refrigerant or Freez Pak Icicle®. Shipments were made by overnight courier and arrived midmorning the following day.

Release sites for *A. citricola* where chosen partly on the basis of a questionnaire which queried growers in the five county area on location, block size, variety, leafminer status, management practices and their willingness to suspend all pesticide applications for one year post-release. In 1994, 1995 and 1996, 13, 39 and 2 releases were made in 10, 25 and 2 groves respectively. Releases were generally made an hour or so before dusk, in trees with ample emerging foliage on which eggs and young Citrus leafminer were present. Vials were opened and wasps allowed to crawl out onto young leaves introduced into the opened neck of the vial to facilitate transfer in the field.

Sampling for evidence of leafminer pupae parasitized by *A. citricola* was conducted at least once, two weeks after each release in 1994 to confirm a first generation. No at-

tempt was made to confirm establishment at 1995 and 1996 releases sites because close proximity to previous release sites would have made it difficult to distinguish between establishment and immigration.

Establishment and Apparent Parasitism

Four 1994 release sites near Immokalee were monitored bi-weekly in 1994 and monthly in 1995 and 1996: Youngquest grove in Lee County, Foundation and Corkscrew groves in Collier County and A. Duda & Sons grove in Hendry County. An additional location, Rosbough grove, directly west of Corkscrew grove was added in late 1994 after *A. citricola* was found to have dispersed there. All groves were sampled for confirmation of establishment and overwintering.

Sampling of *A. citricola* was conducted from time of release/discovery at monitored release blocks through late October when citrus normally becomes dormant (Jackson 1991), and reinitiated in May 1995 and 1996. Samples were obtained by examining 100 intact pupal chambers of citrus leafminer chosen at random or the number encountered in 30 minutes. Pupal chambers were sampled since *A. citricola* and native parasitoids pupate outside the host co-incident with or previous to host pupation. The "sausage link" pupae of *A. citricola* within the host pre-pupal skin of the last host larval instar were easily distinguished from pupae of leafminers or native parasitoids, all of which were noted (Fig. 1). The resulting estimate of parasitoid incidence provided a convenient measure for comparing parasitoid activity among groves (Southwood 1978).

Dispersal

Dispersal was documented by surveying 23 citrus groves for the presence of *A. citricola* outward along 2 transects originating from each of 6 release sites between July and October, 1995. Intact citrus leafminer pupal chambers were examined at all encountered groves for 30 minutes or until *A. citricola* was discovered, whichever came first. Transects were followed until the end of contiguous grove was reached or another release site was encountered and ranged from 1.6-30 miles (2.6-48.3 Km).

Grove structure

Release sites were categorized as either single or multiple aged groves. Multiple aged groves contained blocks of trees consisting of the original planting, usually 15 years or older, along with resets of various ages comprising approximately 50% of the trees. A grove with 60% or more of the trees being uniform in size and age was considered single aged. By these criteria, 6 of the 11 release sites in 1994 were multiple-aged and 5 were single-aged blocks. In 1995, 4 of 17 release sites were multiple-aged and 13 single-aged.

Weather

Weather stations were monitored at two sites; Corkscrew grove and at the University of Florida's Southwest Florida Research and Education Center adjacent to the Foundation grove near Immokalee, Florida. Temperature and humidity (HMP35C probe), wind direction (024A probe) and speed (014B probe) were continuously recorded using a CR10 weather station (Campbell Scientific, Inc. Logan Utah 84321) at both sites in 1995 and 1996.

Fig. 1. Pupae *Pnigalio minio* (Walker) (top), *Ageniaspis citricola* Logvinovskaya (middle), and *Phyllocnistis citrella* Stainton (bottom).

RESULTS

Weather

Climatic conditions for 1995 and 1996 at the research center and 1996 at Corkscrew grove are presented in Table 1. Temperatures at the research center ranged from highs of 36.1°C and 35.5°C in August 1995 and July 1996, to lows of -1.6°C and -2.7°C in February 1995 and 1996 respectfully. Highs at Corkscrew grove reached 36.2°C in September, and dropped to -2.5°C in February. On February 4th, 17th, and 18th, 1996, three freezes were recorded with temperatures at -2.4, -1.9, and -0.9°C respectively at Corkscrew grove, and -2.7, -2.2, and 0°C respectively at the research cen-

TABLE 1. MAXIMUM, MINIMUM AND AVERAGE TEMPERATURE (C°) ALONG WITH AVERAGE RELATIVE HUMIDITY (%) AT SOUTHWEST FLORIDA RESEARCH AND EDUCATION CENTER, IMMOKALEE, FLORIDA IN 1995 AND 1996, AND CORKSCREW GROVE, COLLIER COUNTY, IN 1996.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Southwest Florida Research and Education Center—1995												
Maximum	27.7	30.0	31.1	32.2	35.0	35.0	35.0	36.1	34.4	33.3	31.6	28.8
Minimum	1.6	-1.6	0.0	10.5	17.2	16.6	21.1	21.1	21.6	15.5	5.0	-0.5
Average	16.6	17.2	20.3	23.0	26.6	26.6	27.5	28.0	27.7	25.8	19.7	16.9
Avg RH	80.0	86.2	85.8	87.0	84.4	82.9	82.5	83.2	84.7	87.0	78.7	NA
Southwest Florida Research and Education Center—1996												
Maximum	28.8	30.0	30.5	33.8	32.7	34.4	35.5	34.4	35.0	32.2	31.1	29.4
Minimum	-1.1	-2.7	1.1	7.2	16.6	16.1	20.5	19.4	18.8	11.1	5.5	3.8
Average	16.6	16.4	18.0	21.6	25.5	26.3	28.3	27.7	27.2	24.1	20.7	18.3
Avg RH	80.8	75.7	73.7	74.1	85.0	83.0	80.0	84.2	83.0	84.1	77.6	80.0
Corkscrew—1996												
Maximum	30.7	31.3	35.0	34.0	35.3	34.6	35.8	35.6	36.2	32.7	32.0	30.7
Minimum	10.6	-2.5	1.1	6.2	15.6	14.7	19.0	18.9	18.0	10.5	10.1	2.3
Average	18.8	16.9	17.8	21.3	24.8	25.6	27.3	26.1	26.2	23.4	19.8	17.5
Avg RH	84.5	79.5	77.8	79.7	84.0	88.0	86.4	89.4	86.7	89.0	83.9	84.6

NA, not available.

ter. An additional freeze was recorded at the research center on January 9th dropping temperatures to -1.1°C. No freeze lasted more then 9 hours.

Average relative humidity at the research center ranged from 87% in April to 73.1% in November in 1995 and 85% to 79.9% in April and March respectively in 1996 (Table 1). Average humidity at Corkscrew grove ranged from 79.5% in March to 89.7% in August, 1996.

Establishment

Wasps were often observed to immediately initiate searching movements accompanied by antennation, and to pause over leafminer eggs or small larvae, apparently to oviposit. Recoveries were made at five release sites in two counties during the May, 1995 survey (Table 2). Recoveries ranged from 10 of 25 pupal chambers yielding 29 *A. citricola* pupae to 2 of 25 yielding 6 *A. citricola* pupae. A mean 2.3 (SE = .13, N = 35) *A. citricola* pupae per pupal chamber were found in 1995.

During the May 1996 survey, A. citricola was recovered at 14 of 28 release sites in three counties (Table 2). Four recoveries were made at groves where *A. citricola* had been released in 1994 and recovered in 1995; the remaining 10 recoveries were made at 1995 release sites. Recoveries ranged from 41 of 100 pupal chambers yielding 94 *A. citricola* pupae to 1 of 100 yielding 2 *A. citricola* pupae. Again, a mean 2.3 (SE = .06, N = 145) A. citricola pupae were recovered from parasitized citrus leafminer pupae/ pupal chambers.

Apparent Parasitism

A detectable reproducing population of *A. citricola* was observed at Corkscrew grove 2 weeks after initial release in 1994. Incidence of *A. citricola* increased to 50% within 3 weeks and remained above 10% through 12 October, 1994, climbing to 83% by June 1995 (Fig. 2A). In contrast, parasitization by native parasitoids, in the genera *Pnigalio* and *Horismenus*, peaked at 30% 4 weeks following the release of *Ageniaspis*, and declined to 8% by June 1995.

Incidence of *A. citricola* at the Youngquest grove was documented on 30 May, 1995, at 10%, ultimately peaking at 90% by October 1995. In 1996, parasitism was documented at 20% on 20 May, peaked at 51% in June, falling to 41% by September. Incidence of *Pnigalio* and *Horismenus* fluctuated between 1% and 19% during the same time period, peaking in August, 1995 at 21% (Fig. 2B).

A. citricola was recovered on 12 October 1994, with an incidence of 1% in the Foundation grove, and again on 31 May 1995 at 2%, increasing to 51% by mid October. In 1996, *A. citricola* was recovered on 23 July with an incidence of 9%, and increasing to 50% by mid September. Parasitism by *Pnigalio* and *Horismenus* fluctuated between 3% and 8% during the same time period, with a maximum of 16% observed on August 9, 1995 (Fig. 2C).

A. citricola was first recovered at Duda grove on 28 November, 1994 with an incidence of 1%, increasing to 15% on 19 June, 1995 and peaking at 86% on 19 October, 1995. Parasitism from *Pnigalio* and *Horismenus* fluctuated between 1% and 40% during the same time period with maximum parasitism observed on 28 November 1994 at 40% (Fig. 2D).

A. citricola was discovered on 17 August 1994 at Rosbough grove, 3 months following their initial release at the adjacent Corkscrew grove. This was the first documented case of *A. citricola* dispersal from grove to grove in Southwest Florida. Incidence of *A. citricola* was 7% on August 17, peaking at 26% on October 6, 1994. In-

TABLE 2. RELEASE SITES, GROVE STRUCTURE, NUMBER OF *AGENIASPIS CITRICOLA* RELEASED, AND PARASITISM LEVELS FROM SAMPLES OF 25 AND 100 *PHYLLOCNISTIS CITRELLA* INTACT PUPAL CHAMBERS DURING MAY 1995 AND 1996 SURVEYS OF 1994 AND 1995 RELEASE BLOCKS, RESPECTIVELY.

Grove Structure[1]	Date	Total[2]	*Ageniaspis* 1995/1996[4]	CLM 1995/1996	Native Parasitoids[3] 1995/1996
MA	3 May 94	75	6/0	17/98	2/2
MA	16 May 94, 12 Apr 95	225	0/0	25/100	0/0
MA	10 Aug 94[5]	0	10/10	12/89	2/1
MA	26 Aug 94	200	9/2	11/95	5/3
MA	1, 25 May, 22 Jul 94	515	8/10	16/89	1/1
MA	20, 28 Sep 95	401	0	99	1
MA	19 Jul 95	300	2	82	16
MA	25 Oct 94	109	2/10	21/82	2/8
MA	28 Oct, 17 Nov 94; 22 May 95; 26 Jun 96	1289	0/0	25/100	0
MA	13 Sep 95	500	35	59	6
MA	7 Jul 95	600	4	80	16
SA	28 Feb 95	285	19	67	14
SA	23 Dec 94; 16 Mar, 20 Jul 95	750	0/0	20/95	5/5
SA	1, 17, 22 Mar, 31 May 95	1125	0	99	1
SA	26 Oct 94, 20 Jul 95	287	0/0	25/97	0/3
SA	25 Oct 94	119	0/0	18/100	7/0
SA	25 Apr, 9 Jun, 4 Aug 95	640	41	58	1
SA	17 May 95	100	3	95	2
SA	16 Jun 95	500	0	40	0
SA	21 Jun 95	235	0	40	0
SA	25 Oct 94	136	0/2	25/97	0/1
SA	16 May 95	300	9	87	4

1. MA = Multiple aged grove; SA = Single aged grove.
2. Total number of *Ageniaspis* released at each individual grove from 1994 to 1996.
3. Two most prevalent species Pnigalio and Horismenus.
4. Single numbers indicate that sampling was only conducted in 1996.
5. Date *Ageniaspis* was discovered at Rosbough grove in Lee county.

TABLE 2. (CONTINUED) RELEASE SITES, GROVE STRUCTURE, NUMBER OF *AGENIASPIS CITRICOLA* RELEASED, AND PARASITISM LEVELS FROM SAMPLES OF 25 AND 100 *PHYLLOCNISTIS CITRELLA* INTACT PUPAL CHAMBERS DURING MAY 1995 AND 1996 SURVEYS OF 1994 AND 1995 RELEASE BLOCKS, RESPECTIVELY.

Grove Structure[1]	Date	Total[2]	*Ageniaspis* 1995/1996[4]	CLM 1995/1996	Native Parasitoids[3] 1995/1996
SA	20 Jul, 4 Aug 95	600	1	89	10
SA	15 Aug 95	100	2	98	0
SA	20 Jan, 5, 22, 31 May 95	921	0	94	6
SA	1 Mar 95	150	1	98	1
SA	27 Jun 95	300	0	98	2
SA	28 Jun 95	300	0	99	1

1. MA = Multiple aged grove; SA = Single aged grove.
2. Total number of *Ageniaspis* released at each individual grove from 1994 to 1996.
3. Two most prevalent species Pnigalio and Horismenus.
4. Single numbers indicate that sampling was only conducted in 1996.
5. Date *Ageniaspis* was discovered at Rosbough grove in Lee county.

cidence of *A. citricola* increased over the 1995 season to a peak of 76% by October. High levels of parasitism were again recorded in 1996, reaching 85%. Parasitism from *Pnigalio* and *Horismenus* never exceed 7% and was almost undetectable in 1996 (Fig. 2E).

Grove structure

A. citricola was detected during the 1995 survey in 5 of the 7 multiple-aged release blocks but in none of the 4 single-aged release blocks (Table 2). The parasitoid was recovered at 7 of the 11 multiple-aged blocks and 8 of the 17 single-aged release blocks during the May 1996 survey (Table 2).

Nine groves where parasite establishment was not initially documented received more than one release between 1994 and 1996, 4 were multiple-age, the remaining 6 single-age. Establishment of *A. citricola* occurred in both types of grove structure with the probability of successful establishment being higher in multiple-aged groves (70%) then in single-aged groves (47%).

Dispersal

Dispersal of *A. citricola* was documented from July to October 1995. Maximum dispersal was documented on 25 October from Green Horizon grove in Collier County to Stoney's Citrus Groves, 16.1 miles (25.9 km) to the southwest (Fig. 3). In July, 1996 it was found through casual observation on Marco Island, 30 miles (48.2 km) south measured from Green Horizon Grove. *A. citricola* was recovered at all sample sites during the survey.

DISCUSSION

A. citricola appeared to establish more quickly in multiple aged blocks compared to single-aged blocks. Parasitoid survival may have been favored in multiple-aged

Fig. 2. Percent apparent parasitism of citrus leafminer pupae by *Ageniaspis citricola* and endemic parasitoids at five monitored groves. Samples taken weekly from 25 May to 21 Oct 1994, monthly from 1 May to 24 Oct 1995 and 23 May 1996 to Sep 1996.

blocks by the shelter and a clement microclimate provided by large trees, and a continual source of host material provided by rapidly growing small trees. In comparison, blocks of uniform age and size are characterized by synchronous flush, and a less stable microclimate that appeared to slow establishment of *A. citricola*. Number of releases required before establishment was documented also appeared to be an indicator of the importance of grove structure. Nevertheless, *A. citricola* eventually was established uniformly throughout the region, demonstrating that overall, conditions are favorable for this species.

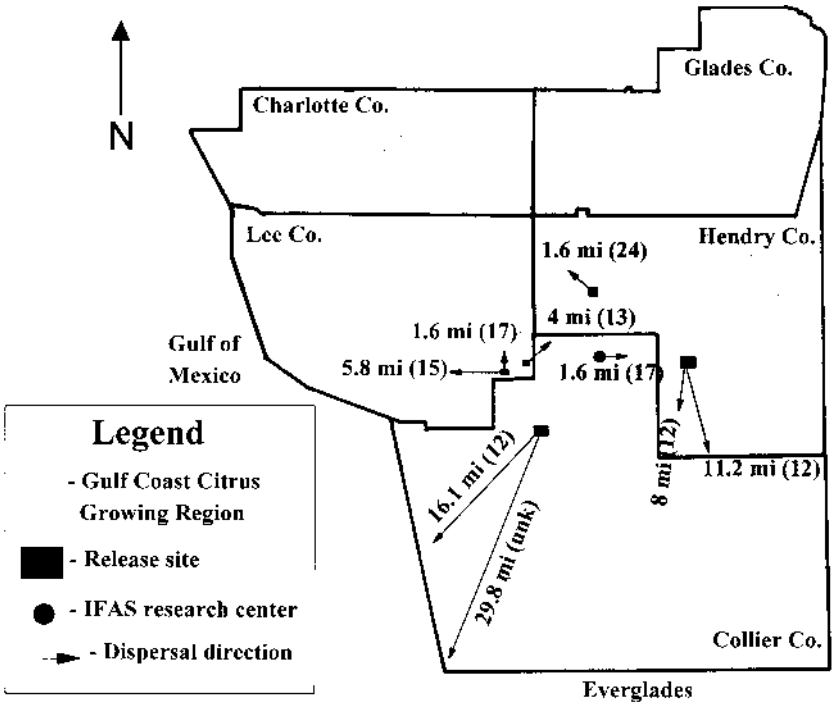

Fig. 3. Dispersal of *Ageniaspis citricola* documented from six release sites in 1996. Relative direction and distance depicted by arrows. Numbers in parenthesis indicate number of months recovery followed release. Arrows and map are not to scale.

These conditions include long growing season with hot, humid summers and mild winters despite two freezes in 1995 and four in 1996. Edwards and Hoy (in press) found that survivorship of *A. citricola* was best at humidities between 80% and 95%, within the average range for Southwest Florida during most of 1995 and 1996 (Table 1). Probability of establishment may have been enhanced by releasing early in the rainy season, May through August, maximizing the interval of warm, humid weather and ideal growing conditions.

Parasitism of citrus leafminer by *A. citricola* steadily increased at most sites following initial release, whereas the proportion of host pupal chambers with native parasitoids tended to decline (Fig. 2). *A. citricola* develops internally, pupates in the leafminer pupal chamber and probably does not have alternate hosts of ecological significance in the area, although there are at least 3 other species of *Phyllocnistis* present (unpublished data). In contrast, native parasitoids of citrus leafminer such as *Pnigalio* spp. etc. are generalists capable of parasitizing many different types of leafminers (Browning et al. 1996, Peña et al. 1996). Also, delayed internal development leaves hosts containing *A. citricola* open to parasitism from quickly developing external native parasitoids such as *Pnigalio* spp, unlikely to distinguish between parasitized and unparasitized citrus leafminer (Pomerinke, personal observation). Yet, in spite of these apparent disadvantages, *A. citricola* came to dominate the nitch provided by citrus leafminer.

Lack of alternate hosts means *A. citricola* populations must track host populations, themselves dependent on the cyclic patterns of flush, which is largely arrested during the winter (Jackson 1991) when generalist native parasitoids would have the greatest advantage. As a likely consequence *A. citricola* was largely absent during the May 1996 samples, whereas native parasitoids where relatively abundant. However *A. citricola* numbers later rebounded, apparently at the expense of native parasitoids. Possibly, the superior host-finding ability that comes with host specificity conferred a key advantage in the competition for hosts.

Superior searching ability was illustrated in the dispersal of *A. citricola* to new groves. In spite of impediments that a small size (approximately 1 mm in length according to Evans 1995 and Logvinoskaya 1983) should pose to directed flight, *A. citricola* was able to disperse over large distances, rapidly colonizing widely scattered citrus plantings over an extensive area (Fig. 3). *Ageniaspis* was found to have dispersed equivalent distances in the Indian river, Homestead, and Orlando citrus growing areas (Hoy et al. 1995). Dispersal distances in Southwest Florida are also consistent with documented dispersal in Louisiana (United States), Bahamas, Honduras, and Australia (Hoy and Nguyen 1997). In this ability *A. citricola* was not unlike its leafminer host which dispersed throughout Florida over a single growing season. Given successful reproduction, overwintering and dispersal throughout Southwest Florida, we feel confident that *A. citricola* has become a permanent addition to the fauna of the region, contributing a significant component of natural mortality to citrus leafminer populations.

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