Contribution of predation and parasitism to mortality of citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) populations in Florida

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Abstract

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton, is native to southern Asia and regarded as an important pest in nurseries and young or top-worked citrus trees in Florida. Damage to the leaf cuticle increases susceptibility to citrus canker disease and further aids spread by increasing inoculum loads. Biological control agents are known to play an important role in regulating pest populations. We evaluated individual contributions of predation and parasitisation to cohorts of *P. citrella* by exclusion and by direct observation of leaf mines in the field. Predation, particularly by ants, was the largest single cause of *P. citrella* mortality, accounting for more than 30% of all deaths by natural enemies, and 60% of all deaths by predators. First and second instars of *P. citrella* were most subject to ant predation. *Ageniaspis citricola* was the most important parasitoid of *P. citrella* and caused 8.2–28.6% mortality compared to 9.6–14.7% from indigenous parasitoids. The total biotic mortality of *P. citrella* observed in exclusion experiments was 52–85%. These results were in basic agreement with 89% mortality, predominantly by predation, obtained by reconstructing a cohort from observations of recently mined leaves. A partial life table based on these data predicted an innate rate of increase (R_o) of 2.8 and thus an almost 3-fold increase per generation. These results indicate that, while biotic mortality takes considerable toll of *P. citrella* populations, the predominance of predation suggests that the parasitoid complex on this exotic pest in Florida is depauperate and would likely be improved by additional introductions.
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1. Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is a pest native to southern Asia that has now spread to all major citrus-growing areas of the world (Hoy and Nguyen, 1997). *P. citrella* was identified from Florida in May 1993 (Heppner, 1993), and spread throughout the peninsula in only one season. Currently, it is regarded as an important pest in citrus nurseries and young or top-grafted trees. Significant impact on growth and yield do not normally occur on trees after four years of age (Browning et al., 2006). However, *P. citrella* is also known to increase the rate of spread of citrus canker disease caused by the bacteria *Xanthomonas citri ex* Hasse by opening the leaf cuticle to infection and increasing the number and severity of lesions, thereby augmenting inoculum (Sohi and Sandhu, 1968; Sinha et al., 1972; Gottwald et al., 1997). The efforts to eradicate citrus canker by removing the infected and neighboring trees in Florida cost the state over 8 million trees before being abandoned in 2006 (http://www.doacs.state.fl.us/pi/canker/).

Biological control has been considered the most economical and environmentally sound long-term solution for managing *P. citrella* in Florida (Knapp et al., 1995). Peña (1994) found that indigenous parasitoids of *P. citrella*

provided low to moderate levels of parasitism in lime orchards in southeast Florida. Peña et al. (1996) reported eight species of indigenous parasitoids of citrus leafminers from Florida. Most (87.4%) of these parasitoids were generalist ectoparasitoids (Hymenoptera: Eulophidae) of late instar larvae of various leafminer species. Among these eulophids, *Pnigalio minio* (Walker) comprised ∼80% of the native parasitoids of *P. citrella* in south Florida. *Ageniaspis citricola* Logvinoskaya (Hymenoptera: Encyrtidae) is an egg-prepupal parasitoid introduced from Australia (Hoy and Nguyen, 1997) that spread quickly in Florida (Pomerinke and Stansly, 1998), soon becoming the most important parasitoid of *P. citrella* in southwest Florida (Pomerinke, 1999). However, in the latter study, predation generally exceeded all other mortality factors including parasitism. A similar study in south Florida found predation to correlate with *P. citrella* density better than all other mortality factors (Amalin et al., 2002).

Quantifying the impact of individual mortality factors on the pest populations has proven to be a major challenge for biological control practitioners and ecologists alike (Luck et al., 1999). Evaluating predation may be especially problematic because many predators remove prey without leaving a particular sign. Fortunately, this is not always the case with leafminers because some predators damage the mines in characteristic ways (Amalin et al., 2002; Grabenweger et al., 2005). Furthermore, when successive observations can be made on the same cohort, mortality may be measured directly (Southwood and Henderson, 2000). Even when more than one mortality factor is acting contemporaneously, a life table can be constructed and marginal attack rates calculated (Elkington et al., 1992).

A variety of predators have been documented to feed on *P. citrella*. Chen et al. (1989) found that a lacewing larva, *Chrysoperla boninensis* Okamoto (Neuroptera: Chrysopidae) could consume ca. 49 *P. citrella* larvae in its lifetime. Huang et al. (1989) concluded that abiotic factors and predation may be two major causes of *P. citrella* mortality during the first and second stadia, because no parasitism was observed during this period. These authors observed ants acting as important predators from late summer to fall during the dry and hot season. Amalin et al. (2002) listed lacewings, ants, hunting spiders and host feeding ectoparasitoids as predators of *P. citrella*. However, they attributed all predation observed in the course of their surveys to spiders and lacewings even though they could not distinguish between these and the other predators listed.

Pomerinke (1999) also reported ants as the major predators on *P. citrella* larvae, contributing an average 33% to total mortality. This estimate was obtained by daily examination of *P. citrella* cohorts on potted citrus plants infested in cages and later placed in citrus orchards. However, recruitment by ants to the concentrated food source thus supplied was not controlled for, either by observing mortality to naturally occurring cohorts or by completely excluding ants for comparison. On the one hand, ant predation may have been overestimated for typical conditions when prey was sparser than that induced on the potted trees. On the other hand, penetration of ants across sticky exclusion barriers could have increased mortality in the controls, resulting in an underestimate of the predation rate. We conducted exclusion experiments, primarily on natural cohorts, to evaluate the relative contribution of biotic factors to *P. citrella* mortality. We also analyzed leafminer survivorship and estimated marginal attack rates from an examination of mines on untreated citrus trees, using the results to construct a virtual cohort with which to estimate innate rate of increase for a *P. citrella* population under natural conditions.

2. Materials and methods

These experiments were carried out in a 28 ha experimental citrus grove at the Southwest Florida Research and Education Center (SWFREC), Immokalee, Florida in 2002 and 2005. The study block consisted of initially five year old sweet orange *Citrus sinensis* (L.) Osbeck 'Valencia' trees planted on double-row raised beds at a density of 323 trees/ha, irrigated by micro-sprinklers and otherwise subjected to conventional cultural practices (Jackson, 1999). A weather station was located within 500 m of the block (http://fawn.ifas.ufl.edu). No insecticides were applied during this study. Where natural infestations of *P. citrella* were insufficient, leaves containing pupae were trimmed from trees and placed into clear 5-cm diameter boxes. After 5–7 days, emerging adults were aspirated out of the boxes into 1.5-cm diameter plastic vials. Adults were fed honey streaked onto tissue paper and allowed to feed overnight. The following day, adults were released into cages confining tree branches containing new expanding leaves, or flushes.

2.1. Mortality factors

Dead and missing *P. citrella* were classified according to cause of death using the following criteria (Pomerinke, 1999; Amalin et al., 2002):

(a) Ants were observed to remove *P. citrella* whole through a small hole in the leaf cuticle over the mine, or through the back of the leaf over the pupal chamber. These signs were considered indicative of ant predation.
(b) Host feeding by parasiotoids of *P. citrella* and predation from other insects was evidenced by part or an entire *P. citrella* cadaver being present in the mine, or by a large and ragged hole in the mine.
(c) Presence of multiple parasitoid pupae within the exoskeleton of the *P. citrella* prepupa and inside the leafminer pupal chamber indicated parasitism by *A. citricola*.
(d) Presence of a larval or pupal parasitoid inside the mine or *P. citrella* pupal chamber indicated parasitism by an indigenous species of Eulophidae.

(e) Physical damage due to rubbing of leaves against other leaves or plant parts was indicated by absence of much of the cuticle over the mine.

2.2. Sticky barrier exclusion

These experiments were conducted to evaluate *P. citrella* mortality from ants and other crawling predators by comparing cohorts protected or not by a sticky barrier (Rudgers and Strauss, 2004). The experimental plants were pruned to induce new flushes to obtain *P. citrella* infestation. Eggs or early first instar larvae were located using a hand lens and identified by marking the adjacent leaf surface with India ink. A sticky barrier (Tangle foot® insect trap coating, The Tanglefoot Company, Grand Rapids, Michigan 49504) was applied to a five centimeter portion of the branch to stop crawling predators from reaching the marked eggs or larvae of *P. citrella*. Branches were monitored daily to confirm that no ants or other predators crossed the sticky barrier. The development stage of *P. citrella* or likely cause of death or disappearance by the criteria above was noted. Observations were made until all individuals had died or emerged. These procedures apply to both experiments described below.

In the first experiment during August, 2002, 14 pruned branches were located on mature citrus trees, each containing expanding leaves and 10–15 *P. citrella* eggs or first instar larvae ready to mine. Branches were randomly divided between control and the sticky barrier treatment. The average minimum and maximum temperatures were 21 and 35 °C, respectively, with rainfall of 11.05 cm during the month.

In a second experiment during June–July, 2005, 480 *P. citrella* eggs or neonate larvae were marked on 40 pruned branches on 20 trees. Branches were randomly divided among control and three treatments with 10 branches (replicates) designated for each treatment. In the control, ants and other crawling predators were allowed to forage normally, whereas three treatments of sticky barrier were timed for 1–13 d, 1–6 d, and 7–13 d, respectively. The experiments were carried out simultaneously on both mature and young orange trees. The average minimum and maximum temperatures were 21 and 34 °C, respectively, with rainfall of 26.11 cm during the two month period.

2.3. Timed cage exclusion

These experiments were conducted to separate the effect of *A. citricola* attacking the egg and early first instar larva of *P. citrella* from native parasitoids and predators attacking later instars. A sleeve cage made of fine mesh organdy was used to exclude entomophaguous insects. The control was no cage rather than an open cage (Smith and DeBach, 1942) so that crawling insects from within the canopy would not be excluded.

In the first experiment during October 2002, 15 mature citrus tree branches containing young expanding leaves (flush) were covered with an organdy sleeve cage as above. A single female of *P. citrella* could deposit 48 (Pandey and Pandey, 1964) to 75 (Margaix et al., 1998) eggs. Male and female pupae were separated according to Jacas and Garrido (1996) and were kept separately in two small tubes for adult emergence at 25 °C. Adults were fed with honey solution for 24 h. Two pairs of adult *P. citrella* were then released in each cage for 48 h to obtain 17–20 eggs per branch for a total of ca. 255–300 eggs. Five branches were randomly selected for each of the two treatments and control: (1) branch caged only from oviposition to day six (= late second instar larvae), (2) branch caged from oviposition until moth emergence, and (3) no cage. Observations were as above. The average minimum and maximum temperatures were 15 and 34 °C, respectively, and rainfall was 21.11 cm during the month.

In the second experiment during July 2005, two pairs of adult *P. citrella* were released in each cage for 48 h to obtain 10–15 eggs per branch. Forty branches were randomly selected for one of the following treatments and control: (1) branch caged from oviposition to day 13, (2) branch caged from early oviposition to day six, and (3) branch caged from day seven until moth emergence; (4) control: no cage. The experiments were conducted both on mature and young citrus trees simultaneously. Other procedure and observations were as above. The average minimum and maximum temperatures were 22 and 34 °C, respectively, with rainfall of 24.45 cm during the month.

2.4. Combined sticky barrier and cage exclusions

This experiment was conducted in September, 2002, to evaluate the separate contributions of predation or parasitism to mortality of later instars of *P. citrella*. Fifteen mature citrus tree branches with natural infestation of *P. citrella* were selected, each containing flush and 15 eggs or neonate larvae that were marked as above. Branches were assigned randomly to one of two treatments or the control, (1) sticky barrier as above, (2) caged branch, and (3) no sticky barrier or cage. The caged branch was covered with an organdy bag 35 cm deep and 15 cm wide, tied closed around the branch from day six (early third instar larva) until adult emergence to exclude predators and indigenous parasitoids. Other conditions and observations were as above. The average minimum and maximum temperature were 22 and 34 °C, respectively, and rainfall was 9.60 cm during the month.

2.5. Effect of caging on hatching rate of P. citrella eggs

Twelve mature citrus tree branches containing young flush were covered with an organdy sleeve cage as above in October, 2002. Several pairs of both genders of adult *P. citrella* were released in the cages for two nights to obtain a total of ca. 250–300 eggs per group (ca. 21–25 eggs per cage). Cages were removed from control branches. All branches were removed after 3–4 days and were examined

in the laboratory under a stereoscopic microscope to count hatched and unhatched eggs. Other conditions were as above.

2.6. Parasitism rates in winter

Mined leaves were collected from 25 randomly selected trees during December 2002 and January 2003. *P. citrella* that had successfully pupated or been parasitized by *A. citricola* or indigenous parasitoids were recorded ($n = 250$). The average minimum and maximum temperatures were 0.56 and 29 °C, respectively, and rainfall was 3.78 cm during the two month period.

2.7. Partial life table

Experimental estimates of *P. citrella* mortality were compared with observations of mined leaves randomly collected from 25 mature trees in the citrus grove on August 8th, 12th, and 14th of 2002. Four hundred mines were examined under a stereoscopic microscope to determine the proportion of *P. citrella* that had survived through emergence or died in different life stages according to the above criteria. Data for stage specific mortality from predation and parasitism were used to construct a partial life table for a reconstructed cohort of *P. citrella* with an initial number set at one hundred. Marginal death rates (m) for predation and parasitism were calculated following the example of a contemporaneous predator and parasitoid given by Elkington et al. (1992). In this example, the predator always "wins" when a host is attacked by both the predator and parasitoid. Therefore, marginal attack rate for predation is same as the death rate and is observed directly, (m predation = d_x predation) while the marginal attack rate for parasitism is m parasitism = d_x parasitism/$(1 - m$ predation).

2.8. Analysis

One-way ANOVA was performed on the data sets from exclusion experiments to evaluate treatment effects (SAS Institute, 1999–2001). Means were separated using Fisher's protected Least Significant Difference (LSD) procedure. Data were arcsine ($\sqrt{P+1}$) transformed for statistical analysis, if necessary (value difference range >40% or data sets with 0 value). However, actual means are reported.

3. Results

3.1. Sticky barrier exclusion

2002. Overall mortality of *P. citrella* averaged more than 80% with no significant effect of the sticky barrier, although there were differences in individual components of mortality (Table 1). Predation accounted for almost 60% mortality in the control compared to half that value in the sticky barrier treatment. In contrast, parasitism accounted for over 40% mortality in the sticky barrier treatment, compared to 18% in the control. Predation was first detected on day three and increased dramatically between days four and five when larvae were still mostly second instars. However mortality by parasitoids was not observed until the late third instar or afterward, so that substantial leaf damage still occurred.

2005. Sticky barriers again did not significantly alter overall mortality of *P. citrella* on mature or young trees or any of the measured components of mortality except for predation by either ants or other predators (Tables 2a,b). Ant predation was reduced both by the early (1–6 d) and complete (1–13 d) barrier treatments but not by the third treatment for which cohorts were protected only during later stages (7–13 d) of *P. citrella* development. Predation by other species was increased by sticky treatments during the entire period or the first 6 days compared to either uncaged branches or branches caged from 7 through 13 d. Thus predation was greatest on early instar prey, and ants either interfered with or masked predation by other species.

3.2. Timed cage exclusion

2002. Caging throughout cohort development significantly reduced overall mortality to a third of that observed

Table 1
Mortality of citrus leafminer cohorts from egg to adult emergence on citrus branches protected with a sticky barrier and on unprotected branches (control), August 2002

Mortality factors		ANOVA	Percentage mortality (mean ± SE)	
		F, (df = 1,12), P	Control	Sticky barrier
Predation	—	39.16, 0.001	57.5 ± 1.2a	28.0 ± 1.5b
	Ants	138.76, 0.001	32.8 ± 1.2a	0b
	Other species	2.37, 0.150	24.7 ± 1.3	28.0 ± 1.9
Parasitism	—	92.98, 0.001	17.8 ± 2.1b	41.2 ± 1.5a
	Ageniaspis citricola	151.54, 0.001	8.2 ± 2.1b	26.5 ± 1.8a
	Other species	4.60, 0.053	9.6 ± 2.1	14.7 ± 2.1
Physical damage	—	3.97, 0.075	9.7 ± 1.8	8.8 ± 2.5
Total mortality	—	2.68, 0.128	85.0 ± 1.5	77.9 ± 1.9

Means in the same row followed by the same or no letter are not significantly different ($P > 0.05$).

Table 2
Mortality of citrus leafminer cohorts from egg to adult emergence on branches of mature and young citrus trees protected with a sticky barrier during 1–13 d, 1–6 d, and 7–13 d of cohort development and on unprotected branches (control), June–July 2005

Mortality factors		ANOVA	Percentage mortality (mean ± SE)			
		F, (df = 3,36), P	Control	Sticky barrier		
				1–13 days	1–6 days	7–13 days
(a) Mature trees						
Predation	—	1.78, 0.168	25.8 ± 1.3	25.0 ± 2.1	27.5 ± 2.5	29.1 ± 1.2
	Ants	241.47, 0.001	15.8 ± 1.7a	0.8 ± 0.2c	7.5 ± 0.9b	15.8 ± 1.4a
	Other species	3.89, 0.017	10.0 ± 1.5b	24.2 ± 2.4a	20.0 ± 2.3a	13.3 ± 1.5b
Parasitism	—	1.34, 0.270	21.6 ± 2.1	28.3 ± 2.5	29.9 ± 2.7	26.7 ± 2.5
	Ageniaspis citricola	0.89, 0.466	8.3 ± 0.9	15.0 ± 1.4	13.3 ± 1.4	14.2 ± 1.5
	Other species	0.750, 0.530	13.3 ± 0.4	13.3 ± 1.2	16.7 ± 1.3	12.5 ± 1.2
Physical damage	—	1.79, 0.167	5.0 ± 1.4	4.2 ± 2.1	5.0 ± 2.1	3.3 ± 0.2
Total mortality	—	1.51, 0.231	52.4 ± 2.5	57.5 ± 3.5	62.4 ± 2.1	59.2 ± 3.1
(b) Young trees						
Predation	—	1.98, 0.134	28.4 ± 1.2	22.5 ± 1.2	29.7 ± 2.6	26.2 ± 2.1
	Ants	308.41, 0.001	16.7 ± 2.3a	0c	5.0 ± 2.4b	14.2 ± 1.3a
	Other species	38.4, 0.001	11.7 ± 1.8b	22.5 ± 1.3a	24.7 ± 2.5a	12.0 ± 1.2b
Parasitism	—	1.86, 0.154	23.3 ± 2.6	28.5 ± 0.5	28.3 ± 2.7	27.5 ± 2.4
	Ageniaspis citricola	0.98, 0.413	11.7 ± 0.9	14.3 ± 2.4	13.3 ± 1.9	14.2 ± 1.3
	Other species	2.6, 0.067	11.6 ± 0.9	14.2 ± 2.1	15.0 ± 0.8	13.3 ± 1.2
Physical damage	—	0.61, 0.613	3.3 ± 0.3	5.0 ± 1.3	6.7 ± 2.4	5.0 ± 0.6
Total mortality	—	2.3, 0.094	55.0 ± 2.3	56.0 ± 2.4	64.7 ± 3.4	58.7 ± 3.2

Means in the same row followed by the same or no letter are not significantly different (LSD, $P > 0.05$).

among cohorts that were not caged, reducing parasitism significantly and totally eliminating predation (Table 3). Overall mortality was significantly reduced in both treatments compared with the control and was significantly lower in the perpetually caged treatment than after only 6 days of caging. Mortality was significantly less than the control for all biotic mortality factors. Predation was greatly reduced by caging during the first 6 days, indicating again that most predation occurred early during cohort development. Parasitism by *A. citricola* was greatly reduced, as expected, by caging either for the first 6 days or during the entire period since this parasitoid oviposits in the host egg. The small amount of parasitism observed on

caged branches probably represents eggs attacked before cage placement. In contrast, eulophid parasitoids were only excluded by the caging during the entire *P. citrella* development period since these species attack late instars. The small amount of parasitism observed on caged branches must represent parasitoids that penetrated the cage or slipped in during observation.

2005. Overall mortality and all biotic mortality was significantly reduced by 1–13 days of caging compared to the control both on mature and young trees (Tables 4a,b). However, early stage (1–6 d) caging significantly reduced total mortality compared with the control only on the young trees (Table 4b). Early stage caging significantly

Table 3
Mortality of citrus leafminer cohorts from egg to adult emergence on citrus branches protected with an organdy cage from egg through day six of larval development or from egg through adult emergence and on unprotected branches (control), October 2002

Mortality factors		ANOVA	Percentage mortality (mean ± SE)		
		F, (df = 2,12), P	Control	Caged from egg through day six of larval development	Caged from egg through adult emergence
Predation	—	194.96, 0.001	21.4 ± 1.5a	5.3 ± 1.6b	0c
	Ants	140.64, 0.001	12.4 ± 1.9a	2.6 ± 2.4b	0b
	Other species	110.60, 0.001	9.0 ± 1.2a	2.7 ± 1.8b	0c
Parasitism	—	71.60, 0.001	39.3 ± 2.3a	28.6 ± 4.6b	13.7 ± 3.4c
	Ageniaspis citricola	67.30, 0.001	16.8 ± 1.6a	5.2 ± 1.1b	9.6 ± 2.0b
	Other species	87.40, 0.001	22.5 ± 1.6a	23.4 ± 1.8a	4.1 ± 1.5b
Physical damage	—	1.54, 0.202	5.6 ± 1.2	3.9 ± 0.3	6.8 ± 0.7
Total mortality	—	127.08, 0.001	66.3 ± 3.1a	37.8 ± 3.7b	20.5 ± 2.8c

Means in the same row followed by the same or no letter are not significantly different (LSD, $P > 0.05$).

Table 4
Mortality of citrus leafminer cohorts from egg to adult emergence on branches of mature and young citrus trees protected with an organdy cage barrier during 1–13 d, 1–6 d, and 7–13 d of cohort development and on unprotected branches (control), July 2005

Mortality factors		ANOVA	Percentage mortality (mean ± SE)			
		F, (df = 3,36), P	Control	Cage barrier		
				1–13 days	1–6 days	7–13 days
(a) Mature trees						
Predation	—	108.16, 0.001	26.8 ± 1.2a	0c	20.1 ± 1.3b	23.7 ± 1.2ab
	Ants	51.80, 0.001	15. 6 ± 0.6a	0c	6.1 ± 1.2b	7.9 ± 0.8ab
	Other species	41.30, 0.001	11.2 ± 1.2a	0b	14.0 ± 0.4a	15. 8 ± 1.5a
Parasitism	—	103.90, 0.001	20.8 ± 2.6ab	1.7 ± 0.5c	15.8 ± 0.4ab	26.3 ± 2.4a
	Ageniaspis citricola	32.60, 0.001	9.1 ± 1.2a	0b	6.2 ± 0.6ab	12.3 ± 2.4a
	Other species	10.48, 0.006	11.7 ± 2.2a	1.7 ± 0.5b	9.6 ± 1.2ab	14.02 ± 1.4a
Physical damage	—	1.39, 0.260	5.8 ± 0.3	5.8 ± 0.1	5.0 ± 0.3	6.13 ± 0.8
Total mortality	—	98.40, 0.001	53.4 ± 2.1a	7.5 ± 0.4b	40.9 ± 2.3a	56.1 ± 3.2a
(b) Young trees						
Predation	—	98.60, 0.001	28.3 ± 1.2a	0c	19.2 ± 1.2b	25.0 ± 2.6a
	Ants	49.30, 0.001	15.8 ± 0.8a	0b	9.2 ± 0.3ab	8.3 ± 0.8ab
	Other species	45.60, 0.001	12.5 ± 0.6a	0c	10.0 ± 2.3b	16.7 ± 1.2a
Parasitism	—	6.40, 0.004	20.0 ± 2.6a	0c	15.8 ± 2.2b	25.0 ± 2.3a
	Ageniaspis citricola	4.1, 0.013	9.2 ± 1.2a	0b	7.5 ± 1.4ab	11.7 ± 1.2a
	Other species	3.1, 0.039	10.8 ± 0.3a	0b	8.3 ± 0.4b	13.3 ± 1.2a
Physical damage	—	0.89, 0.260	3.8 ± 0.3	6.7 ± 3.2	4.1 ± 1.5	6.7 ± 0.5
Total mortality	—	103.40, 0.001	52.1 ± 2.3a	6.7 ± 3.2c	39.1 ± 2.2b	56.7 ± 2.4a

Means in the same row followed by the same or no letter are not significantly different (LSD, $P > 0.05$).

reduced predation on both mature and young trees, however, parasitism was reduced only on young trees but the trends were similar to those observed above.

3.3. Combined sticky barrier and cage exclusions

Both the sticky barrier and caging from day six reduced all types of predation, although parasitism was increased by the sticky barrier (Table 5). Apparent parasitism by *A. citricola* was also increased by late stage caging whereas parasitism by other species was unaffected by either treatment. This latter result was unexpected since the cages should have excluded the parasitoids. The effect on *A. citricola* could possibly be explained by ant predation on parasitized

leafminers. In any case, the overall result was no significant effect of either exclusion treatment on total mortality due to compensating effects of individual biotic mortality factors.

3.4. Effect of caging on hatching rate of P. citrella eggs

Percentage hatching of *P. citrella* eggs did not differ significantly ($F = 2.5$; df $= 1,10$; $P > 0.05$) between caged ($75.0 ± 1.9$) and uncaged ($68.1 ± 2.3$) branches.

3.5. Parasitism of P. citrella in winter

Parasitism of *P. citrella* prepupae by *A. citricola* from 8th December 2002 to 16th January 2003 averaged

Table 5
Mortality of citrus leafminer cohorts from egg to adult emergence on citrus branches protected with a sticky barrier or an organdy cage placed at day six of cohort development and on unprotected branches (control), September 2002

Mortality factors		ANOVA	Percentage mortality (mean ± SE)		
		F, (df = 2,12), P	Control	Sticky barrier	Caged from day six
Predation	—	30.49, 0.001	43.1 ± 2.5a	18.6 ± 1.5b	14.6 ± 1.0b
	Ants	10.87, 0.003	21.5 ± 0.9a	0c	9.4 ± 1.1b
	Other species	14.87, 0.001	21.6 ± 1.8a	18.6 ± 1.5a	5.2 ± 0.4b
Parasitism	—	6.49, 0.012	24.5 ± 3.2b	47.1 ± 5.6a	37.5 ± 3.2ab
	Ageniaspis citricola	6.21, 0.014	10.7 ± 0.6b	28.6 ± 1.3a	20.3 ± 1.0a
	Other species	2.1, 0.162	13.8 ± 1.2	18.5 ± 0.8	17.2 ± 1.1
Physical damage	—	3.1, 0.082	9.2 ± 0.2	5.6 ± 0.3	12.1 ± 0.8
Total mortality	—	1.03, 0.301	76.8 ± 3.5	71.4 ± 5.6	64.2 ± 3.1

Means in the same row followed by the same or no letter are not significantly different (LSD, $P > 0.05$).

$57 \pm 12.2\%$, whereas parasitism by indigenous species averaged only $7.7 \pm 5.01\%$.

3.6. Partial life table

Apparent mortality observed directly by examination of mines was estimated to range from 31% to 42% for the first three larval instars, predominantly from predation (Table 6). Apparent mortality to the combined prepupal and pupal stage was estimated at 56% and due entirely to parasitism, of which 73% was attributed to *A. citricola*. By then the cohort had been reduced by 75%, so that the contribution of parasitism to mortality of the original cohort was only 14%. Survival to adulthood was estimated at 11%, with predation accounting for slightly less than 60% of real mortality and parasitism slightly less than 30%.

4. Discussion

Both predation and parasitism contributed significantly to the mortality of *P. citrella*. However, predation, predominantly by ants acting on early instars of *P. citrella* was the largest single apparent cause of mortality accounting for over 30% of all deaths by natural enemies, and 60% of all deaths by predators. These results corroborated those of Pomerinke (1999) and Amalin et al. (2002). Huang et al. (1989) also observed ants acting as important predators of *P. citrella* in China, especially in late summer to fall during the dry and hot season. However, Urbaneja et al., 2004) did

not see a significant reduction in predation on *P. citrella* in Spain by attempting to exclude ants using a trunk treatment of insecticidal dust. This exclusion was directed particularly at *Lasius niger* (Latreille), a ground nesting species. Pomerinke (1999) observed nine ant species foraging in potted citrus trees infested with *P. citrella*. However, only two of these, *Pseudomyrmex gracilis* (Roger), and *Crematogaster ashmeadi* (Mayr), were actually seen feeding on *P. citrella*. These two are arboreal species nesting in hollow stems and twigs (King, 2004) and would probably be not be excluded by insecticidal trunk treatment.

Ant predation was observed to occur by removal of the *P. citrella* larva through a small hole made in the mine. Nevertheless, we could not ascertain that ants were responsible for all such damage because the actual act of predation was rarely witnessed. Amalin et al., 2002) also recorded greater mortality to *P. citrella* populations from predation than from parasitism, scoring the latter as having been caused by spiders and lacewings, even though they were unable to distinguish between these and other predators. Spiders are known to feed on *P. citrella* in two different ways, either through the mine and leaving a cadaver as do lacewings or host feeding hymenopter, or removing the prey through slit made in the mine as do ants (Amalin et al., 2001, 2002).

Spiders could drop down or balloon onto a branch so would not be as well excluded by a sticky barrier as would ants. Therefore, the fact that sticky barriers resulted in a significant decline in the incidence of mortality characterized

Table 6
Partial life table calculated according to Elkington et al. (1992) for a reconstructed cohort of citrus leafminer based on mortality to different life stages as determined by examination of mines during August 2002

Stage	Factor	l_x	Stage d_x[b]	Factor d_x[c]	Marginal Death rate m[d]	Apparent mortality Stage $q_x = d_x/l_x$	Apparent mortality Factor $q_x = d_x/l_x$	Real mortality Stage d_x/l_0[a]	Real mortality Factor d_x/l_0[a]
1st instar larva		100[a]	31.4			0.314		0.314	
	Predation			31.4	0.314		0.314		0.314
	Parasitism			0.0	0.000		0.000		0.000
2nd instar larva		69	28.7			0.416		0.287	
	Predation			19.8	0.198		0.287		0.198
	Parasitism			8.9	0.111		0.129		0.089
3rd instar larva		40	15.0			0.375		0.150	
	Predation			8.4	0.084		0.210		0.084
	Parasitism			6.9	0.075		0.173		0.069
Prepupa and pupa		25	14.0			0.560		0.140	
	Predation			0.0	0.000		0.000		0.000
	Parasitism			14.0	0.140		0.560		0.140
Total					0.922				0.894
	Predation				0.596				0.596
	Parasitism				0.326				0.298
Adult		11							

[a] Initial number entering the first stage in the life table (l_0).
[b] Number dying in each stage.
[c] Factor contribution to stage mortality.
[d] m predation $= d_x$ predation, m parasitism $= d_x$ parasitism / ($1 - m$ predation).

by a hole in the mine and absence of a leafminer cadaver supports ants as the main cause of this type of predation. Furthermore, most predators not excluded by sticky barriers left a partial or entire prey cadaver. A trend for increased non-ant predation and parasitism behind sticky barriers could indicate interference by ants with other predators. Indeed, antagonistic behavior of ants toward other natural enemies has been reported from numerous other systems, though generally when the host is a honeydew source (Buckley, 1987). However, consumption by ants of parasitized leafminers and partially consumed cadavers could also explain our results. Future experiments should be designed to distinguish between these two possibilities.

Real mortality to the entire cohort up to 85% was observed during the course of exclusion experiments, mostly attributable to predation. These results were corroborated by mine analysis which showed an estimated 89% mortality; of which close to 60% was attributed to predation and close to 30% to parasitism when marginal rates were calculated for individual contributions of both factors acting contemporaneously (Table 6). The relative importance of predation compared to parasitism in the population dynamics of *P. citrella* was in contrast to the norm of parasitoid dominance in the population dynamics of leafminers in general (Hawkins et al., 1997). The relatively minor contribution of all parasitoids except the introduced *A. citricola* may be another indication of a depauperate parasitoid complex attacking *P. citrella* in Florida. Furthermore, given an average fecundity of 75 eggs per female (Margaix et al., 1998), fertility of 68.1% as obtained on uncaged leaves, a sex ratio of 1:1 and survivorship 11% ($l_x = 0.11$) in the immature stages, we could estimate an innate rate of increase (R_o) for *P. citrella* equal to $(11/100) \times 0.5 \times 75 \times 0.68 = 2.8$ females per generation, (Bellows and Van Driesche, 1999). The potential for an almost 3-fold increase in each generation is another indication that additional sources of mortality are needed to maintain control of this pest.

Ageniaspis citricola is maladapted to dry conditions that occasionally predominate in Florida, especially during the critical early growing season. Adult *A. citricola* are short-lived, susceptible to desiccation, and require high humidity to survive (Yoder and Hoy, 1998). For this reason, *A. citricola* has done well when introduced into humid regions such as Florida (Hoy and Nguyen, 1997), Brazil (Nogueira de Sá et al., 2000) and Queensland (Neale et al., 1995). It has not performed well in dry climates such as the Mediterranean (Argov and Rössler, 1996; Michelakis and Vacante, 1997; Mineo and Mineo, 1999; Garcia-Mari et al., 2004). Several recent years of drought conditions in Florida have had a negative effect on *A. citricola* populations, resulting in extended lag times between leafminer and parasitoid populations. Thus, it would be desirable to import, evaluate and release drought-tolerant parasitoid species to complement the control provided by *A. citricola* and other natural enemies of *P. citricola* in Florida.

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