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Exclusion experiments reveal relative contributions of natural enemies to mortality of citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Alabama satsuma orchards

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ABSTRACT

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is an important pest of satsuma mandarin (*Citrus unshiu* Marcovitch) in Alabama. A recent study identified several species of beneficial arthropods including spiders (e.g., *Hibana* sp.), ants (e.g., *Solenopsis invicta* Buren), *Chrysoperla* sp., *Harmonia axyridis* Pallas, and two parasitoid species (*Ageniaspis citricola* Logvinovskaya and *Cirrospilus* sp.), as potential natural mortality factors of *P. citrella* in Alabama. Exclusion experiments with complete or timed (early or later) sticky or cage barriers were conducted in two satsuma orchards in southern Alabama during 2007 and 2008 to determine the relative contributions of key beneficial arthropods to natural mortality of *P. citrella*. Overall mortality of *P. citrella* on unprotected (control) satsuma tree branches ranged from ~39%–52% depending on location and year. Results from both locations and years clearly showed that predation was the dominant natural mortality factor acting on *P. citrella*. Predation accounted for ~87–96% of all deaths on unprotected (control) satsuma tree branches. In particular, predation by spiders was the single most important mortality element, which accounted for ~50–70% of all deaths. Predation by ants was second, accounting for ~10–19% of all deaths. Predation by predatory insect larvae accounted for ~3–27% of all mortalities, while parasitism contributed the least (0–10%) to *P. citrella* mortality. Predation by spiders was excluded by a cage barrier, whereas a sticky barrier was more effective in excluding predation by ants. In general, the timed barrier treatments were not as effective in excluding the key predatory arthropods. These results are discussed in relation to the management of *P. citrella* in Alabama satsuma orchards.

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1. Introduction

Satsuma mandarin (*Citrus unshiu* Marcovitch) production is a growing industry in southern Alabama and other parts of the Gulf Coast region of the United States (Campbell et al., 2004). Recent surveys have identified the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) as a key pest of this specialty fruit crop in Alabama (Fadamiro et al., 2007, 2008). *P. citrella* originated from southeast Asia and has become a global pest of citrus, having been found in Africa, Australia, the Middle East, the Caribbean, Central, South, and North America, (Heppner, 1993; Heppner and Dixon, 1995; Pena et al., 1996; Perales-Gutierrez et al., 1996; Hoy and Nguyen, 1997; Legaspi et al., 1999; Diez et al., 2006). In the United States, *P. citrella* was first recorded in 1993 in citrus nurseries in Dade County, Florida (Heppner, 1993). The pest is

now found throughout the state of Florida, as well as in several other citrus producing states including Alabama, Louisiana, Texas and California (Legaspi et al., 1999; Gill, 1999).

P. citrella attacks all varieties of citrus, other Rutaceae plants, and several ornamental species (Heppner, 1993; Legaspi et al., 1999). Females lay eggs on the leaves of host tree and eclosing larvae feed on the leaf epidermis ingesting sap and causing chlorosis and curled leaves (Heppner, 1993; Legaspi et al., 1999). Larvae of *P. citrella* make characteristic serpentine mines under the leaf cuticle, which may reduce photosynthesis (Cook, 1988). The feeding tunnels produced by *P. citrella* larvae on citrus leaves may facilitate infection by the citrus canker bacterium, *Xanthomonas axopodis* pv. *citri* (Sohi and Sandhu, 1968; Cook, 1988; Gottwald et al., 1997, 2002). High population densities of *P. citrella* are usually recorded in spring and summer due to greater availability of leaf flushes and new shoots, as well as higher temperatures (Pena et al., 1996; Legaspi et al., 1999; Diez et al., 2006). *P. citrella* is an important pest in citrus nurseries and top-grafted trees (Diez et al., 2006), and heavy infestation can have significant impact on growth and yield (Pena et al., 2000; Browning et al., 2006).

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Control of *P. citrella* is typically accomplished through multiple applications of conventional insecticides, which are often ineffective because the larvae are usually concealed within the mines and thus are protected from insecticide sprays (Legaspi et al., 2001). Biological control is generally regarded as the most economically sound and environmentally sustainable management practice for *P. citrella* (Knapp et al., 1995; Hoy and Nguyen, 1997). The population dynamics of *P. citrella* and associated natural enemies have been documented in several countries and regions (Chen et al., 1989; Pena et al., 1996; Pena, 1998; Urbaneja et al., 2000; Legaspi et al., 2001; Diez et al., 2006; Lapointe and Leal, 2007). Several predatory arthropods are known to feed on *P. citrella*, including lacewing larvae, ants, and hunting spiders (Argov and Rössler, 1996; Pomerinke, 1999; Amalin et al., 2001a,b; Hoy et al., 2007; Xiao et al., 2007), and many studies have identified predation as the most important natural mortality factor acting on *P. citrella* in many parts of the world (Chen et al., 1989; Amalin et al., 1996, 2001a,b, 2002; Hoy et al., 2007; Xiao et al., 2007). In addition, many species of parasitoids have been reared from *P. citrella* worldwide (Hoy and Nguyen, 1997; Schauff et al., 1998; Legaspi et al., 1999), however, indigenous parasitoids were found to provide only minimal levels of parasitism in Florida (Pena et al., 1996). Consequently, *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae), an exotic specialist endo-parasitoid of *P. citrella* larvae, was introduced in the 1990s for classical biological control of the pest in Florida and Texas (Hoy and Nguyen, 1997; Pomerinke and Stansly, 1998).

In a recent study (Xiao and Fadamiro, in review), we documented the natural enemy fauna of *P. citrella* in southern Alabama as consisting of at least 21 species of beneficial arthropods, including various species of spiders (e.g., *Hibana* sp., *Cheiracanthium* sp. and *Hentzia* sp.) (Araneae), ants (Hymenoptera: Formicidae), *Chrysoperla* spp. (Neuroptera: Chrysopidae), and *Harmonia axyridis* (Coleoptera: Coccinellidae). Two parasitoid species were also detected in low numbers: *A. citricola* and *Cirrospilus* sp. (Hymenoptera: Eulophidae). *Cirrospilus* spp. are indigenous generalist ectoparasitoids of *P. citrella* in the United States (Pena et al., 1996; Schauff et al., 1998). However, little is known about the impact of these beneficial arthropods on *P. citrella* in Alabama. In this study, exclusion techniques (e.g., Smith and DeBach, 1942; Xiao et al., 2007; Qureshi and Stansly, 2009) were used to determine the relative contributions of key beneficial arthropods to natural mortality of *P. citrella* in Alabama satsuma orchards. The results should provide the baseline data necessary for development of an effective biological control program for managing *P. citrella* in the Gulf Coast region of the United States.

2. Materials and methods

2.1. Study sites

Experiments were conducted in July 2007 and August 2008 in two satsuma orchards located in Baldwin County in southern Alabama: Coker orchard and the Gulf Coast Research and Extension Center orchard (GGREC). Both orchards were comprised mainly of satsuma mandarin, with a few sweet orange (*Citrus sinensis*), grapefruit (*C. paradise*), and kumquat (*Fortunella* spp.). The predominant cultivar of satsuma mandarin was 'Owari', with few trees each of 'Armstrong Early' and 'Brown's Select'. Both orchards were typically managed using conventional practices including routine applications of pesticides, but were not sprayed during this study.

2.2. Exclusion experiments

Experimental trees in both orchards were first pruned to induce new flushes necessary for *P. citrella* infestation. The flushes were

observed daily using a hand lens to determine peak period of *P. citrella* egg hatch. Newly hatched first instar larvae (one day old) were then located using a hand lens and identified by marking the adjacent leaf surface with dark ink. Only one first instar larva was kept per leaf, the remaining larvae and/or eggs were removed by hand. Two types of exclusion techniques (sticky barrier and cage barrier) were used to evaluate the relative contribution of natural enemies to *P. citrella* mortality, following the procedures described by Xiao et al. (2007) and Qureshi and Stansly (2009), but with some minor modifications. The use of both exclusion techniques was timed in order to determine the impact of various biotic factors attacking larvae of *P. citrella* at the different development stages. The aim of the sticky barrier exclusion treatments was to evaluate *P. citrella* mortality factors due to ants and other crawling predators (e.g., lacewing and lady beetle larvae) by comparing mortalities in *P. citrella* cohorts protected by a sticky barrier versus unprotected (control) cohorts. The cage barrier exclusion treatments were tested to separate the effect of other biotic factors (e.g., spiders and parasitoids) attacking larvae of *P. citrella*.

In the first experiment (July 2007), 30 pruned branches located on mature satsuma trees were selected in each orchard. Each branch contained expanding leaves and 15 first instar larvae of *P. citrella*. The branches were randomly distributed among the following six treatments (i.e. each treatment was replicated five times): (i) branch protected with sticky barrier from days 1 to 12 (sticky barrier 1–12 or complete sticky barrier); (ii) branch protected with sticky barrier from days 1 to 5 with the sticky barrier removed on day 6 when the larvae had become mostly late second instars (sticky barrier 1–5 or early sticky barrier); (iii) branch protected with sticky barrier from days 6 to 12 when the larvae had become pupae (sticky barrier 6–12 or later sticky barrier); (iv) branch caged from days 1 to 5 with the cage removed on day 6 when the larvae had become mostly late second instars (cage barrier 1–5 or early cage barrier); (v) branch caged from days 6 to 12 when the larvae had become pupae (cage barrier 6–12 or later cage barrier); (vi) control (branch not protected with sticky barrier or cage throughout the experiment).

Our use of the terms "early" versus "late" application of exclusion barriers (sticky or cage) was based on preliminary tests which showed that that most larvae at the end of day 5 were late second instars, and on day 6 were mostly early 3rd instars. Thus, we considered application of exclusion barriers from days 1 to 5 as "early" since the larvae were first to second instars, and from days 6 to 12 as "late" since the larvae were mostly third instars and older. For the sticky barrier treatments, a 5-cm diameter sticky trap strip (Trécé Inc., Salinas, CA) was applied to the branch to preclude ants and other crawling predators from reaching the larvae. For the cage barrier treatments, a sleeve cage made of fine mesh organdy (35 × 15 cm diameter, white color made from screen netting) was used to exclude beneficial arthropods, such as spiders and parasitoids. The control (no sticky or cage barrier) ensured that no arthropods were excluded (Smith and DeBach, 1942).

The experiment was repeated in August 2008 but with some minor modifications. At each location, 28 pruned branches, each containing expanding leaves and 15 newly hatched first instar larvae of *P. citrella*, were randomly distributed among seven treatments (i.e. four replicates per treatment). The treatments consisted of the six treatments evaluated in 2007 plus a seventh treatment, a branch caged from days 1 to 12 (cage barrier 1–12 or complete cage barrier). The average temperatures at both locations during the experiments in July 2007 and August 2008 were 29.3 °C (minimum: 24.5 °C, maximum: 34 °C) and 28.0 °C (minimum: 23.4 °C, maximum: 32.5 °C), respectively. Average rainfall (precipitation) for July 2007 and August 2008 was 5.12 and 5.33, respectively.

2.3. Evaluation of mortality factors

Branches were inspected daily noting and recording the development and survival of the larvae, until all larvae had died or emerged. The number of dead larvae and the likely cause of the death were also noted. Pupae were checked for parasitism starting from day 10. Dead and missing larvae were classified according to the likely cause of death using the following criteria (Pomerinke, 1999; Amalin et al., 2002; Xiao et al., 2007): (a) Spiders: spiders were observed to puncture immobile larvae over mines, sucking the body fluid or making a slit in the mine to remove larvae; (b) Ants: ants were observed to remove larvae from the mine through a small hole in the leaf cuticle, or through the back of the leaf over the pupa chamber; (c) Other predators (e.g., lacewing and lady beetle larvae) were observed to feed on body parts of *P. citrella* larvae without removing the entire cadaver (this category also included undetermined predation); (d) Parasitism by ecto-parasitoids (e.g., *Cirrospilus* spp.): presence of larval or pupal parasitoid inside the mine or pupal chamber of *P. citrella*; (e) Parasitism by endo-parasitoids, (e.g., *A. citricola*): presence of multiple parasitoid pupae within pupal chamber of *P. citrella*; and (f) Physical mortality (including mortality due to abiotic factors): absence of much of the cuticle over the mine.

2.4. Statistical analysis

Daily survivorship was calculated for larvae in the different treatments. Percentage cumulative larval mortality (at the end of the experiment) was calculated for each treatment (by mortality factor) and used for statistical analysis. Data obtained were first normalized by using the arcsine square-root transformation ($\sqrt{x + 0.5}$) and then analyzed with one-way analysis of variance (ANOVA) followed by the Tukey–Kramer honestly significant difference (HSD) test to determine significant treatments effects ($P < 0.05$, JMP Version 7.01, SAS Institute, 2007). Data for each orchard and year were analyzed and presented separately.

3. Results

Daily survivorship of *P. citrella* larvae in the control and key exclusion treatments evaluated in 2007 and 2008 are shown in Figs. 1 and 2. As expected, survivorship of larvae on unprotected (control) branches was lower than larval survival in the complete sticky barrier and cage exclusion treatments in which larvae were protected for the entire duration of the study. In general, significant differences in larval survival in the control versus exclusion treatments were recorded beginning as early as days 2 or 3 of

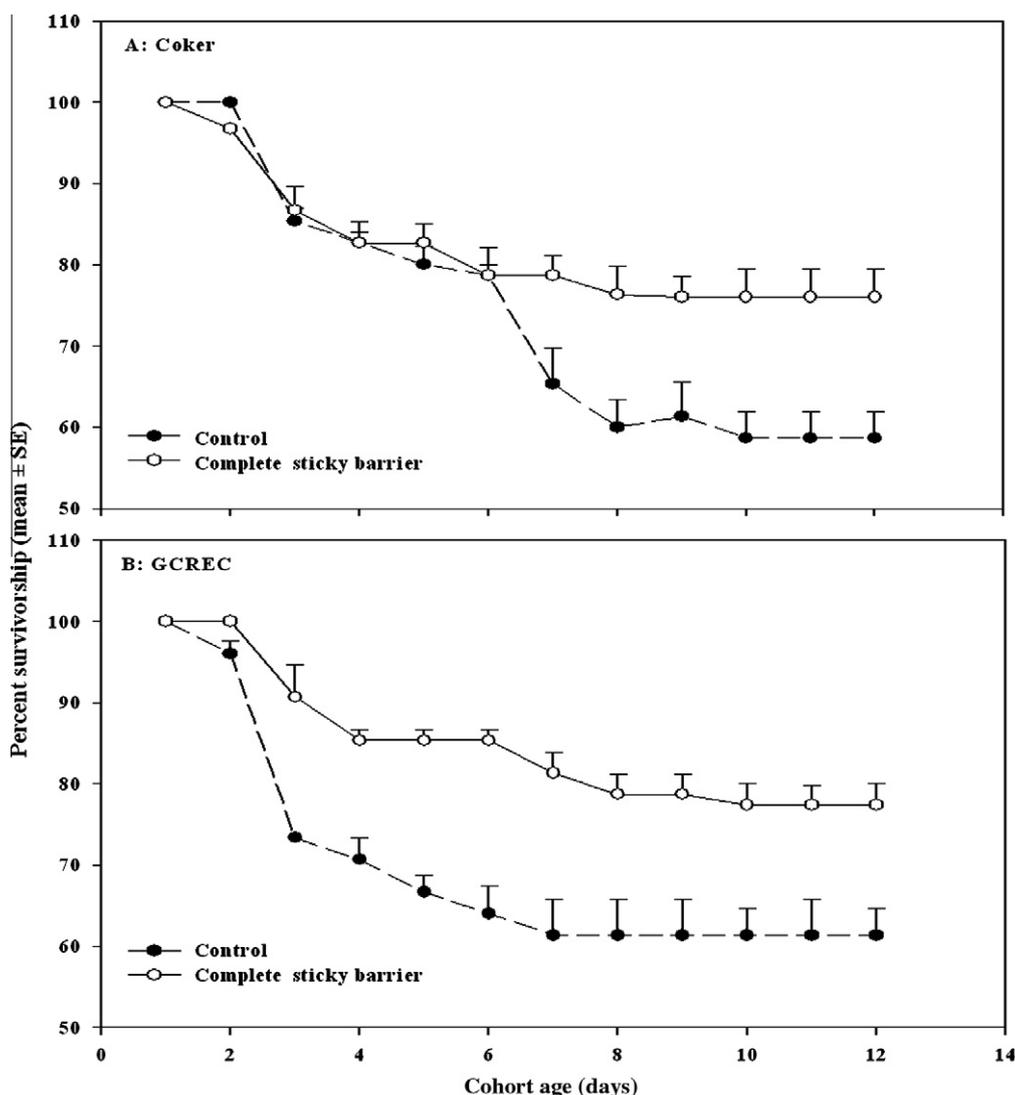


Fig. 1. Survivorship curve of *P. citrella* immature stage over whole development periods in natural and protected condition in the two orchards in 2007. Figure showed mean (\pm SE) numbers of *P. citrella*. (A) Coker and (B) GCREC (Gulf Coast Research and Extension Center, Fairhope, AL).

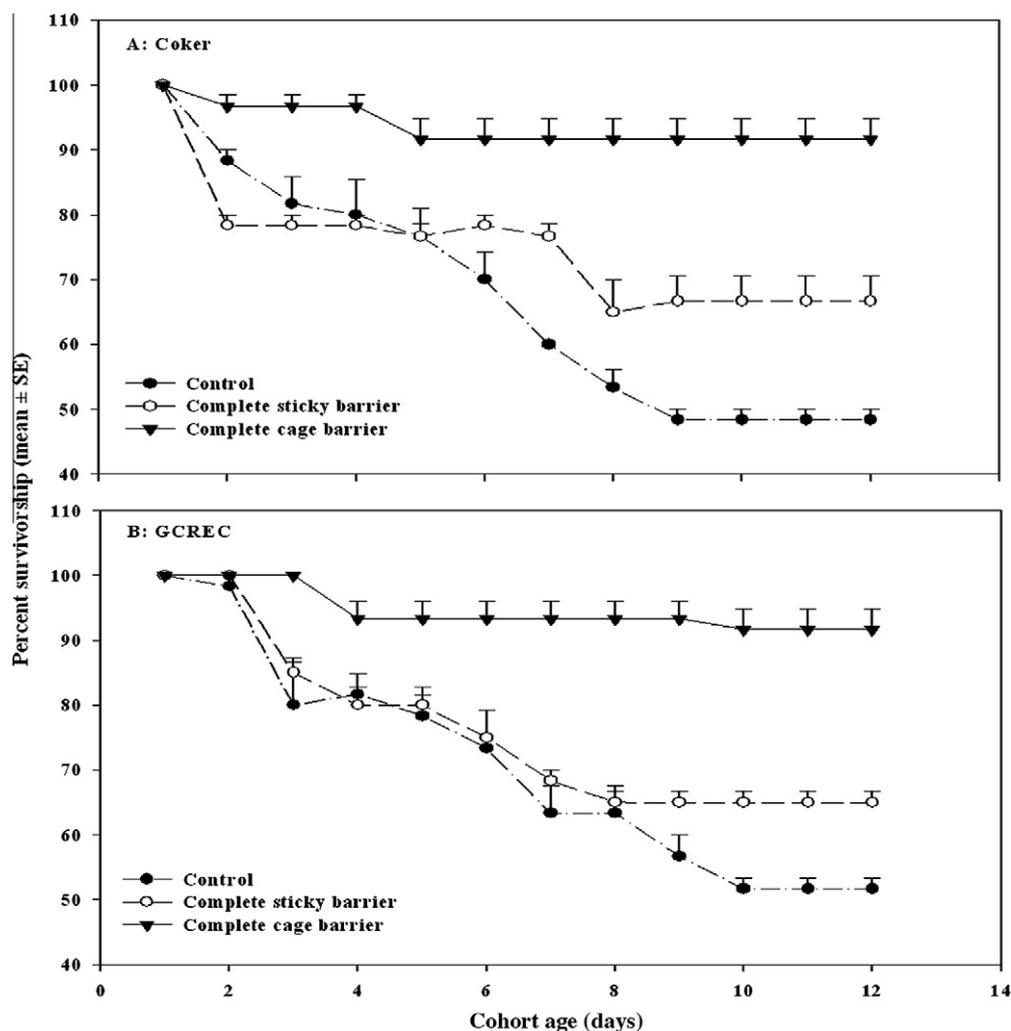


Fig. 2. Survivorship curve of *P. citrella* immature stage over whole development periods in natural and protected condition in the two orchards in 2008. Figure showed mean (\pm SE) numbers of *P. citrella*. (A) Coker and (B) GCREC (Gulf Coast Research and Extension Center, Fairhope, AL).

the experiment. In 2007 at Coker orchard, however, similar larval survival rates were recorded from days 1 to 6 of the experiment in the control versus the complete (1–12 days) sticky barrier treatment, after which larval survival in the control began to reduce significantly (Fig. 1A). The complete (1–12 days) cage barrier treatment was evaluated in 2008, which allowed for a comparison between the two complete exclusion (sticky barrier versus cage barrier) techniques. At both locations, daily larval survivorship was significantly greater in the complete cage barrier treatment than in the complete sticky barrier treatment beginning from day 2 until the end of the experiment (Fig. 2). For instance, percent survival of larvae on day 12 in the complete cage barrier treatment was $\sim 92\%$ in both orchards compared with $\sim 65\text{--}67\%$ in complete sticky barrier treatment and $<50\%$ in the control. These results suggest that the cage exclusion method was more effective in excluding key natural enemies, and consequently in reducing larval mortality.

Percentage cumulative mortalities of *P. citrella* larvae recorded at the end of the experiment (12 days) in the different treatments are presented in Tables 1–4. In general, significant differences in mortalities were recorded among the treatments, with the highest mortalities recorded in the control. Overall mortality in the control was $\sim 39\text{--}41\%$ in 2007 (Tables 1 and 2) and $\sim 48\text{--}52\%$ in 2008 (Tables 3 and 4) in both orchards. In both orchards in 2007 (Tables 1 and 2), predation by spiders was the single most impor-

tant mortality factor accounting for $\sim 25\text{--}30\%$ mortality, whereas parasitism accounted for only $\sim 1\text{--}3\%$ mortality in the control. All five exclusion (sticky barrier and cage barrier) treatments significantly reduced predation by spiders, which also resulted in significant reductions in overall larval mortalities compared with the control. However, the effects of the other mortality factors (i.e. ants, other predators and physical damage) were not significantly different between the control and any of the exclusion treatments. Furthermore, no significant differences in total larval mortalities were recorded among the five exclusion treatments, irrespective of whether the larvae were protected early (1–5 days), later (6–12 days) or throughout the experiment (1–12 days). Comparison among the three sticky barrier treatments showed that only the complete (1–12 days) sticky barrier treatment was very effective in excluding ants.

In 2008 at Coker orchard, spiders also were the most important mortality factor which accounted for $\sim 25\%$ mortality in the control. In contrast, predation by spiders was completely excluded (0%) in the complete (1–12 days) cage barrier treatment, and significantly reduced ($\sim 13\%$) in the later (6–12 days) cage barrier treatment, but not significantly reduced in the other treatments compared with the control (Table 3). Similarly, predation by ants and other predators were completely (0%) excluded in the complete (1–12 days) cage barrier treatment. Predation by ants was also completely excluded (0%) in the complete (1–12 days) sticky

Table 1

Mortality of *Phyllocnistis citrella* cohorts from first instar larvae to adult emergence on unprotected (control) branches of satsuma trees versus branches protected with different exclusion techniques (sticky barrier or cage) during different stages of development at Coker orchard in 2007.

| Exclusion treatments | Percentage mortality (mean ± SE) due to each mortality factor | | | | | |
|-------------------------|---|-------------------------|-----------------------|-----------------------|----------------------|------------------------|
| | Spiders | Ants | Other predators | Parasitoids | Physical | Total mortality |
| Control | 29.3 ± 4.9a | 6.7 ± 2.1 | 1.3 ± 1.3 | 2.7 ± 1.6 | 1.3 ± 1.3 | 41.3 ± 5.7a |
| Complete sticky barrier | 13.3 ± 1.6b | 0 ± 0 | 6.7 ± 2.1 | 1.3 ± 1.3 | 2.7 ± 1.6 | 24.0 ± 3.4b |
| Early sticky barrier | 13.3 ± 0b | 1.3 ± 1.3 | 5.3 ± 1.6 | 1.3 ± 1.6 | 1.3 ± 1.6 | 22.7 ± 1.6b |
| Later sticky barrier | 9.3 ± 1.6b | 2.7 ± 1.6 | 4.0 ± 1.6 | 2.7 ± 1.6 | 2.6 ± 1.6 | 21.3 ± 2.5b |
| Early cage barrier | 9.3 ± 1.6b | 1.3 ± 1.3 | 4.0 ± 1.6 | 4.0 ± 1.6 | 2.7 ± 1.6 | 21.3 ± 1.3b |
| Later cage barrier | 10.7 ± 1.6b | 4.0 ± 2.6 | 1.3 ± 1.3 | 1.3 ± 1.6 | 2.7 ± 1.6 | 20.0 ± 3.0b |
| ANOVA (F, P) | F = 10.7, df = 5, 24 | F = 1.96, P = 0.0001 | F = 1.81, P = 0.14 | F = 0.24, P = 0.94 | F = 0.2, P = 0.95 | F = 4.99, P = 0.002 |

Means in the same column followed by the same or no letter are not significantly different ($P > 0.05$, Tukey–Kramer HSD test). In this and subsequent tables, complete sticky barrier = branch protected with sticky barrier from days 1 to 12; early sticky barrier = branch protected with sticky barrier from days 1 to 5; later sticky barrier = branch protected with sticky barrier from days 6 to 12; complete cage barrier = branch caged from days 1 to 12; early cage barrier = branch caged from days 1 to 5; later cage barrier = branch caged from days 6 to 12; and control = branch not protected with sticky barrier or cage. Other predators = other crawling predacious insects (e.g., lacewing and lady beetle larvae).

Table 2

Mortality of *Phyllocnistis citrella* cohorts from first instar larvae to adult emergence on unprotected (control) branches of satsuma trees versus branches protected with different exclusion techniques (sticky barrier or cage) during different stages of development at GCREC orchard in 2007.

| Exclusion treatments | Percentage mortality (mean ± SE) due to each mortality factor | | | | | |
|-------------------------|---|-----------------------|-----------------------|----------------------|-----------------------|------------------------|
| | Spiders | Ants | Other predators | Parasitoids | Physical | Total mortality |
| Control | 25.3 ± 2.5a | 4.0 ± 1.5 | 5.3 ± 1.3 | 1.3 ± 1.3 | 2.7 ± 1.3 | 38.6 ± 3.2a |
| Complete sticky barrier | 14.7 ± 2.5b | 0 ± 0 | 5.3 ± 1.3 | 0 ± 0 | 2.7 ± 1.3 | 22.7 ± 2.6b |
| Early sticky barrier | 12.0 ± 1.5b | 4.0 ± 1.6 | 8.0 ± 2.5 | 0 ± 0 | 2.7 ± 1.3 | 26.7 ± 3.6b |
| Later sticky barrier | 10.0 ± 1.6bc | 2.7 ± 1.6 | 8.0 ± 1.3 | 5.3 ± 2.5 | 2.7 ± 1.1 | 28.7 ± 2.6b |
| Early cage barrier | 9.3 ± 2.6b | 2.7 ± 1.6 | 5.3 ± 1.3 | 0 ± 0 | 2.7 ± 1.3 | 20.0 ± 2.9b |
| Later cage barrier | 8.0 ± 1.3bc | 2.7 ± 1.6 | 5.3 ± 2.5 | 2.7 ± 2.6 | 4.0 ± 1.6 | 22.7 ± 3.4b |
| ANOVA (F, P) | F = 10.4, df = 5, 24 | F = 0.96, P = 0.46 | F = 0.58, P = 0.71 | F = 1.0, P = 0.44 | F = 0.11, P = 0.98 | F = 4.46, P = 0.005 |

Means in the same column followed by the same or no letter are not significantly different ($P > 0.05$, Tukey–Kramer HSD test). GCREC = Gulf Coast Research and Extension Center, Fairhope, AL.

Table 3

Mortality of *Phyllocnistis citrella* cohorts from first instar larvae to adult emergence on unprotected (control) branches of satsuma trees versus branches protected with different exclusion techniques (sticky barrier or cage) during different stages of development at Coker orchard in 2008.

| Exclusion treatments | Percentage mortality (mean ± SE) due to each mortality factor | | | | | |
|-------------------------|---|-------------------------|------------------------|-----------------------|-----------------------|--------------------------|
| | Spiders | Ants | Other predators | Parasitoids | Physical | Total mortality |
| Control | 25.3 ± 1.7a | 9.7 ± 1.8ab | 10.0 ± 1.3ab | 5.0 ± 3.2 | 1.6 ± 0.8 | 51.6 ± 1.7a |
| Complete sticky barrier | 21.7 ± 1.7a | 0 ± 0c | 8.3 ± 1.7ab | 3.3 ± 0.5 | 0 ± 0 | 33.3 ± 3.8b |
| Early sticky barrier | 18.3 ± 1.7ab | 1.7 ± 0.8 | 13.3 ± 1.9a | 3.3 ± 3.3 | 1.7 ± 0.9 | 38.3 ± 3.2ab |
| Later sticky barrier | 18.3 ± 2.3ab | 10.0 ± 1.6ab | 3.3 ± 1.9bc | 5.0 ± 1.7 | 0 ± 0 | 36.6 ± 3.3b |
| Complete cage barrier | 0 ± 0c | 0 ± 0c | 0 ± 0c | 1.6 ± 0.5 | 6.7 ± 2.7 | 8.3 ± 3.2c |
| Early cage barrier | 15.0 ± 1.7a | 0 ± 0c | 3.3 ± 1.3bc | 6.7 ± 1.9 | 3.3 ± 1.9 | 28.3 ± 7.4b |
| Later cage barrier | 13.3 ± 1.7b | 6.7 ± 2.7bc | 5.0 ± 0.5ab | 0 ± 0 | 1.7 ± 0.8 | 26.7 ± 2.7b |
| ANOVA (F, P) | F = 47.7, df = 6, 21 | F = 12.5, P = 0.0001 | F = 6.03, P = 0.009 | F = 0.69, P = 0.65 | F = 1.87, P = 0.13 | F = 11.05, P = 0.0001 |

Means in the same column followed by the same or no letter are not significantly different ($P > 0.05$, Tukey–Kramer HSD test).

barrier treatment. Parasitism was generally low ranging from 0% in the later cage barrier treatment to ~5% in the control. Compared with the control, overall mortality was significantly reduced in four of the five exclusion treatments, with the early (1–5 days) sticky barrier treatment being the exception. This suggests that the effect of predation by ants was greatest on early instar larvae. Among the three cage barrier treatments, the complete cage barrier treatment was the only treatment which effectively excluded spiders and ants (Table 3). No consistent differences were recorded between the early and the later cage barrier treatments, suggesting that *P. citrella* larvae are susceptible to predation by spiders and ants throughout their development. In general, similar results were recorded at the GCREC orchard: overall mortality was significantly

reduced in five of the six exclusion treatments. The lowest mortality (~8%) was recorded in the complete cage barrier treatment compared with 35% mortality in the complete sticky barrier treatment and 48% mortality in the control (Table 4).

4. Discussion

The results from both locations and years clearly showed that predation was the most significant biological interaction, which accounted for ~87–96% of all *P. citrella* larval mortalities in unprotected (control) trees in Alabama satsuma orchards. In particular, predation by spiders was the single most important natural mortality factor acting on early (1st–2nd) and late (3rd and older)

Table 4
Mortality of *Phyllocnistis citrella* cohorts from first instar larvae to adult emergence on unprotected (control) branches of satsuma trees versus branches protected with different exclusion techniques (sticky barrier or cage) during different stages of development at GCREC orchard in 2008.

| Exclusion treatments | Percentage mortality (mean ± SE) due to each mortality factor | | | | | |
|-------------------------------|---|------------------|-------------------|------------------|------------------|-------------------|
| | Spiders | Ants | Other predators | Parasitoids | Physical | Total mortality |
| Control | 25.0 ± 3.2a | 8.3 ± 3.2a | 13.3 ± 2.3a | 0 ± 0 | 1.7 ± 1.7 | 48.3 ± 1.7a |
| Complete sticky barrier | 20.0 ± 2.7a | 0 ± 0b | 10 ± 1.9ab | 3.3 ± 1.9 | 1.7 ± 1.7 | 35.0 ± 1.7ab |
| Early sticky barrier | 18.3 ± 1.7a | 3.3 ± 1.9b | 5.0 ± 1.7bc | 0 ± 0 | 3.3 ± 1.9 | 29.9 ± 3.3b |
| Later sticky barrier | 18.3 ± 1.7a | 6.7 ± 0a | 6.7 ± 2.7bc | 0 ± 0 | 0 ± 0 | 31.7 ± 3.2b |
| Complete cage barrier | 0 ± 0b | 0 ± 0b | 0 ± 0c | 3.3 ± 1.9 | 5.0 ± 1.7 | 8.3 ± 3.2c |
| Early cage barrier | 16.7 ± 1.9a | 8.3 ± 1.7a | 8.3 ± 1.7ab | 0 ± 0 | 0 ± 0 | 33.3 ± 2.7b |
| Later cage barrier | 18.3 ± 1.7a | 6.7 ± 2.7a | 3.3 ± 1.9bc | 0 ± 0 | 1.7 ± 1.7 | 30.0 ± 4.3b |
| ANOVA (<i>F</i> , <i>P</i>) | <i>F</i> = 14.4, | <i>F</i> = 4.18, | <i>F</i> = 6.68, | <i>F</i> = 2.5, | <i>F</i> = 1.5, | <i>F</i> = 15.5, |
| <i>df</i> = 6, 21 | <i>P</i> = 0.0001 | <i>P</i> = 0.006 | <i>P</i> = 0.0005 | <i>P</i> = 0.054 | <i>P</i> = 0.225 | <i>P</i> = 0.0001 |

Means in the same column followed by the same or no letter are not significantly different ($P > 0.05$, Tukey–Kramer HSD test). GCREC = Gulf Coast Research and Extension Center, Fairhope, AL.

instars of *P. citrella*. The key predacious spider species recorded in our study included the yellow glost spider, *Hibana* sp. (Anyphaenidae), the long-legged sac spider, *Cheiracanthium* sp. (Miturgidae), and *Hentzia* sp. (Salticidae). Several authors have also reported on the role of spiders and insect predators as a key or dominant natural mortality factor acting on *P. citrella* in many parts of the world (Amalin et al., 1996, 2001a,b, 2002; Browning and Pena, 1995; Argov and Rössler, 1996; Xiao et al., 2007; Hoy et al., 2007). Three species of sac spiders, *Chiracanthium inclusum* (Hentz) (Clubionidae), *Hibana velox* (Becker) (Anyphaenidae), and *Trachelas volutes* Gertsch (Corrinnidae) were reported to feed on *P. citrella* larvae and pre-pupae in Florida lime orchards (Amalin et al., 1996, 2001a,b). Further studies on their predatory habit showed that they are nocturnal species with the ability to detect their concealed prey by sensing movements (vibrations) of *P. citrella* larvae and pre-pupae with the leaf epidermis (Amalin et al., 2001a,b). Prey preference studies also confirmed that certain predacious spider species prefer to feed on Lepidopteran and Homopteran pests in orchards (Jackson, 1977; Nyffeler et al., 1987; Amalin et al., 2001a,b; Brown et al., 2003; Stephen and Berg, 2008). Spiders are known to feed on *P. citrella* in two ways. They may directly puncture mines or remove the prey through open slit in the mines (Amalin et al., 2001a,b; Xiao et al., 2007). Thus, the specialized predatory habit and feeding behavior of spiders make them important predators of *P. citrella* and similar pests. In addition to directly feeding on the immature stages, the webs spun over citrus leaves by spiders may make the leaves less suitable for oviposition and feeding by pests (Stephen and Berg, 2008).

Predation by ants was the second most important mortality factor, which accounted for ~10–19% of all *P. citrella* deaths on unprotected (control) branches. The key ant species recorded included *Solenopsis invicta* Buren and *Brachymyrmex patagonicus* Mayr, and both species were observed feeding on *P. citrella* larvae. Ants have also been reported as important predators of *P. citrella* in many parts of the world (Huang et al., 1989; Pomerinke, 1999; Amalin et al., 2001a,b; Xiao et al., 2007), but their impact appears to vary by region or climate. For instance, ants were the key predators of *P. citrella* in China, in particular during the dry and hot summer and fall seasons (Huang et al., 1989). Also, predation by ants, such as *Pseudomyrmex gracilis* (Roger) and *Crematogaster ashmeadi* (Mayr), was the largest single cause of *P. citrella* mortality which accounted for >30% of all mortality by natural enemies in a south-west Florida citrus grove (Xiao et al., 2007). In contrast, Urbaneja et al. (2004) reported no significant effect of ant exclusion on mortality of *P. citrella* in Spain. Ants have been observed to remove *P. citrella* larvae through a small hole made in the mine, resulting in missing cadavers (Amalin et al., 2001a,b; Pomerinke, 1999; Xiao et al., 2007). Predation by other predatory insects, such as larvae

of lacewings (*Chrysoperia* sp.) and the multicolored Asian lady beetle (*H. axyridis*) accounted for ~3–27% of all *P. citrella* mortalities, as has been reported by other authors (Chen et al., 1989; Amalin et al., 2002).

Two important parasitoids of *P. citrella*, *Cirrospilus* sp. and *A. citricola*, were recorded in this study. *Cirrospilus* spp., are indigenous generalist ecto-parasitoid of *P. citrella* in the US (Pena et al., 1996; Schauff et al., 1998), while *A. citricola* is an introduced parasitoid of *P. citrella* in Florida and Texas (Hoy and Nguyen, 1997; Pomerinke and Stansly, 1998). However, parasitism contributed only minimally (~0–10%) to *P. citrella* mortality in the present study. Several authors have reported low to moderate rates of parasitism of *P. citrella* larvae in the field (Pena et al., 1996; Legaspi et al., 2001; Amalin et al., 2002; Diez et al., 2006; Xiao et al., 2007). Legaspi et al. (2001) recorded ~20% parasitism in Mexico, with the dominant parasitoid being *Zagrammosoma multilineatum* (Ashmead) (Hymenoptera: Eulophidae). The introduced parasitoid, *A. citricola* was the dominant parasitoid recorded in southwest Florida, and accounted for ~8–29% of *P. citrella* natural mortality (Xiao et al., 2007). We are not aware of any purposeful introduction of *A. citricola* in Alabama, and its occurrence is likely due to accidental introductions through movement of rootstock materials from Florida or Texas, or range expansion by natural dispersal. The relatively minor impact of parasitism recorded in the present study may simply be a reflection of the low endemic population densities of the identified parasitoids in Alabama citrus orchards. Since *A. citricola* has been reported to perform well in humid regions (Neale et al., 1995; Hoy and Nguyen, 1997; Xiao et al., 2007), field augmentation of this parasitoid species is likely to be successful in the humid southern Alabama conditions.

The sticky barrier and cage barrier exclusion methods evaluated in this study have been commonly used to assess the effectiveness and impact of natural enemies in the field (Grabenweger et al., 2005; Pomerinke, 1999; Xiao et al., 2007; Qureshi and Stansly, 2009). Our results which showed that predation by spiders was completely excluded in the complete cage barrier treatment, and reduced in the complete sticky barrier treatment, are not surprising given their behavior on trees (Stephen and Berg, 2008). Spiders could move from branch to branch by ballooning and thus would not be that affected by a sticky barrier. As expected, predation by ants was excluded or significantly reduced in the complete sticky barrier treatment. Interestingly, predation by ants was also excluded or reduced in the cage barrier treatment. Similarly, key predatory larvae were also excluded or reduced in the complete cage barrier treatment but the numbers were sometimes too low to detect a significant effect. These results possibly indicate that the mesh size of the cage barrier was fine enough to exclude some ants (e.g., major ant workers) and predatory larvae. Cage barriers

may influence larval development by providing suitable microclimate for the larvae. However, this was not supported by our results since most individual larvae in either caged, sticky or control treatments had progressed to the pupal stage by day 12 of the experiment. This is in support of a previous study which also showed that cage exclusion did not significantly accelerate *P. citrella* egg hatch and development (Xiao et al., 2007). In general, the timed (early or later) barrier treatments were not as effective in excluding or reducing any of the key predatory arthropods. It is not surprising that parasitism by *A. citricola* was not excluded in the complete cage barrier treatment given that this species is a parasitoid of egg and early instar larvae of *P. citrella* (Hoy and Nguyen, 1997). Use of a cage barrier starting from newly deposited eggs would have provided a more appropriate exclusion technique for *A. citricola*. Nevertheless, this design flaw did not appear to have any significant impact on our results given the generally low parasitism rates recorded in both orchards. A recent study also confirmed the generally low occurrence of *A. citricola* in Alabama citrus orchards (Xiao and Fadamiro, unpublished data).

In summary, our results showed that predation by spiders and ants are very important natural mortality factors acting on *P. citrella* in Alabama citrus. Conservation of these key predators through the judicious use of pesticides and augmentation of field populations of key natural enemies are central to the development of a sustainable pest management strategy for the pest in Alabama.

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