Predation by Hunting Spiders on Citrus Leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)[1]

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Abstract Feeding efficiency of the three species of hunting spiders, *Chiracanthium inclusum* Hentz, *Hibana velox* (Becker), and *Trachelas volutus* (Gertsch), was investigated in laboratory, greenhouse, and field-cage experiments. Results of the laboratory feeding efficiency tests showed that the predation rates of these hunting spiders can be ranked as follows: *H. velox* > *C. inclusum* > *T. volutus.* A similar trend of predation rates was obtained from the feeding efficiency test under greenhouse conditions. The predation efficiency of *H. velox* was further tested in a field-cage experiment because it was found to be a more efficient predator than *C. inclusum* and *T. volutus* from the laboratory and greenhouse feeding efficiency tests. The results of the field-cage experiment indicated that as the population density of the citrus leafminer increases, the number of citrus leafminer consumed by *H. velox* also increases. In addition, significantly more citrus leafminer larvae were consumed when two to three *H. velox* were present inside the cage than when only one *H. velox* was present.

Key Words *Phyllocnistis citrella, Chiracanthium inclusum, Hibana velox, Trachelas volutus, Citrus aurantifolia,* citrus leafminer, hunting spiders, sac spiders, lime, biological control

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is one of the major pests of *Citrus* species. The species was first described in Calcutta, India (Stainton 1856) and is considered native to south and southeast Asia (Schauff and La Salle 1996). This Asian species is widely distributed and found in many countries in the world where citrus is grown (Heppner 1993). The impact of this pest has prompted intensive studies on its biology and population dynamics. Eggs are deposited individually in the adaxial or abaxial sides of young leaves. The eggs hatch in 2 to 10 d depending on the temperature (Knapp et al. 1995). Upon hatching, the larvae feed in the leaf parenchyma and produce serpentine mines beneath the leaf epidermis where they are protected during their feeding cycle. Pupation occurs in the leaf. The citrus leafminer is multivoltine in southern Florida, and the total generation time can fluctuate between 13 to 52 d depending on the temperature (Peña et al. 1996).

Biological control appears to be the most promising tactic for the management of the citrus leafminer. Several species of natural enemies have been evaluated against this pest (Heppner 1993, Zhang et al. 1994, Argov and Rossler 1996). Worldwide, various parasitoids of the citrus leafminer have been identified. For instance, in South-

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east Asia alone, 58 species of parasitoids have been associated with the citrus leafminer (Heppner 1993, Ujiye et al. 1996). In Florida, at least six species of native parasitoids have been found since the arrival of the citrus leafminer (Browning and Peña 1995, Browning et al. 1996). Moreover, two species of parasitic wasps, *Ageniaspis citricola* Logvinovskaya and *Cirrospilus ingenuus* Gahan (Hoy and Nguyen 1994, as *C. quadristiatus*) have been introduced into Florida from Australia to control the citrus leafminer. These parasitoids are already established and now are an important addition to the already existing complex of parasitoids and predators attacking the citrus leafminer in Florida (Hoy and Nguyen 1994, LaSalle et al. 1999).

Experimental evidence shows the important contribution of predatory arthropods to the mortality of the citrus leafminer in the field (Zhao 1989, Zhang et al. 1994, Chen et al. 1989, Browning 1994, Argov and Rossler 1996). In south Florida, several predators such as lacewings, *Chrysoperla rufilabris* (Burmeister), flower bugs, *Orius insidiosus* (Say), ants, and various species of spiders were considered important in reducing peak population of the citrus leafminer (Browning and Peña 1995, Amalin et al. 1996, Peña and Subramanian, unpubl. data).

Few studies have identified indigenous generalist spiders (Amalin et al. 1996) or assessed their effects on citrus leafminer population densities. Survey results in lime orchards in south Florida revealed that the most dominant predatory arthropods are the hunting spiders (Amalin 1999). The abundance of this group of spiders in lime orchards probably means that their preferred prey is also abundant in the area. The same group of spiders was reported abundant in unsprayed apple orchards and found attacking the spotted tentiform leafminer (Lepidoptera: Gracillariidae) (Wisniewska and Prokopy 1993, Corrigan and Bennett 1987). This species of leafminer belongs to the same family as the citrus leafminer. It seems that this group of spiders may tend to specialize on leafminer pests. Thus, our interest in investigating the potential of hunting spiders as one of the mortality factors acting on citrus leafminer in lime orchard was heightened. This present study was conducted to evaluate the efficiency of the three most dominantly occurring species of hunting spider: *Chiracanthium inclusum* Hentz, *Hibana velox* (Becker), and *Trachelas volutus* (Gertsch), in lime orchards as predators of citrus leafminer under laboratory, greenhouse, and field cage experiments.

Materials and Methods

Laboratory studies of feeding efficacy. Laboratory reared second- and third-nymphal stages of the three species of hunting spiders-*C. inclusum, H. velox,* and *T. volutus*-were placed individually in a 15 mm high × 100 mm diam Petri dish lined with a gel substrate (400 mg agar, dissolved in 1000 ml of distilled water, with 2 mg Benlate dissolved in 20 ml acetone and autoclaved at 1.1 kg cm^{-2} for 15 min). Portions of leaf containing 10 second-instar *P. citrella* larvae were placed in each Petri dish. The containers were kept in an incubator maintained at 27°C, 12L:12D, and 80% RH. During 3 consecutive days, the larvae were removed daily from the containers and replaced with new ones. Mortality of *P. citrella* was recorded every 3 days for 2 wks. The experiment was repeated 3 times on 3 different dates. The percent citrus leafminer mortality by different spiderling nymphal stages was noted for each species, and the mean differences were compared using Duncan's Multiple Range Test (DMRT).

Greenhouse studies of feeding efficacy. Predation of *C. inclusum, H. velox,* and *T. volutus* on *P. citrella* larvae was measured under greenhouse conditions using branch cages. The cages consisted of modified 2-liter soft drink bottles made of clear polyethyleneterethylate. The two ends of the bottle were cut to produce a 240 × 120 mm tube. A fabric sleeve was attached to each open end of the tube. One branch of a lime plant growing in a 2-liter pot was enclosed in each cage. All branches used in this study had 15.0 ± 1.3 leaves to produce a standard infestation of 20 first- and second-instar larvae of *P. citrella* per cage. All other species of insects or mites were hand-removed from the cage. This was done to keep the area of discovery constant during the experimental period and to prevent other factors from interfering with the parameters being examined.

A single third nymphal stage spider was placed into each cage, and dead citrus leafminer larvae were counted 1 wk after exposure. Control cages contained *P. citrella* but no spiders. Each treatment was replicated three times. The temperature fluctuated daily from 25 to 27°C with 70.0% ± 9.6 RH. Mean weekly predation rates were recorded and compared between prey regimes by one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) (SAS 1989).

Field cage studies of feeding efficacy. Research was conducted during July and August 1998, at the Tropical Research and Education Center, Homestead, FL. Rates of predation of *H. velox* on first, second, and third larval instars and on the prepupal stage of *P. citrella* were measured using field cages. Cages were constructed of nylon mesh screen (91-cm-wide × 91-cm-long × 122-cm-high) supported on PVC frames. One side of the cage had a slit opening provided with a zipper to close and open the cage. The cages enclosed 2-yr-old potted lime plants. All plants were previously infested with citrus leafminer to produce a standard infestation per plant. Only first, second, and third instars were retained, and all other *P. citrella* stages removed. This was done to keep the prey numbers constant throughout the experiment. Before the plant was introduced into the cage, the plants were shaken vigorously to dislodge any resident arthropods, which were then removed. Each pot inside the cage was placed on a plastic pot container with water. This was done to prevent other predators (i.e., ants) from gaining access through the bottom slit of the cage.

Two different experiments were conducted to determine spider predation rates. For the two experiments, fourth nymphal stage of *H. velox* previously fed with artificial diet (Amalin et al. 1999) were used. In the first experiment, 0, 1, 5, 10, and 25 *P. citrella* larvae were placed in each cage in which a single spider was added. Each treatment was replicated three times. Control cages contained prey but no predators. In the second experiment, 25 first and second instars of *P. citrella* were placed in each cage and 1, 2, and 3 fourth nymphal stages *H. velox* were introduced separately into each cage. Cages were inspected twice a week for 2 wks. Dead citrus leafminer were recorded and removed. Control cages contained prey but no predators. Each treatment was replicated three times. Percent mortality was calculated as number of dead citrus leafminer divided by the total number of citrus leafminer in each plant. Mean weekly predation rates were compared between predator regimes by analysis of variance, and differences among treatments were compared by Duncan's Multiple Range Test (SAS 1989).

Results and Discussion

Feeding efficiency tests. Results of the feeding efficiency tests under laboratory conditions are shown in Table 1. The second nymphal stage *H. velox* and *T. volutus*

Table 1. Percent citrus leafminer larval mortality caused by second- and third-nymphal stages of the three species of hunting spiders under laboratory feeding efficiency tests

Spider species	Percent citrus leafminer mortality by different spiderling nymphal stages	
	Second nymphal stage	Third nymphal stage
Chiracanthium inclusum	28.5 b	43.5 b
Hibana velox	58.3 a	83.3 a
Trachelas volutus	56.3 a	39.7 b

Means followed by the same letters within a column are not significantly different ($P \leq 0.05$) according to DMRT.

consumed significantly more citrus leafminer larvae than *C. inclusum.* The third nymphal stage *H. velox* consumed significantly more citrus leafminer larvae than corresponding third nymphal stage *C. inclusum* and *T. volutus* (Table 1). The predation rates of the three species of hunting spiders on citrus leafminer larvae under greenhouse conditions showed similar trends to those obtained from the laboratory efficiency test (Fig. 1). For both feeding efficiency tests, the predation rate of the three species of hunting spiders can be ranked as follows: *H. velox* > *C. inclusum* > *T. volutus.* This finding suggests that *H. velox* is a more efficient predator than *C. inclusum* or *T. volutus.* Nevertheless, it is possible that the presence of these 3 species of spiders could give a higher degree of predation on citrus leafminer population than a single predator in the orchard. Riechert and Lawrence (1997) reported that a spider assemblage performed two times better in limiting prey than did any given predatory species by itself. It is, therefore, worthwhile to evaluate the predation effects of the assemblage of the three species of hunting spiders on citrus leafminer population.

The result of the feeding trial using field cage experiment indicated that as the density of citrus leafminer increases, the number of citrus leafminer consumed by *H. velox* also increases (Table 2). There was a significant correlation [$r^2 = 0.994$; $y = 0.675 + 0.502\,(x)$] between citrus leafminer density and the number of citrus leafminer consumed by the spider (Fig. 2). Feeding trials on other species of spiders showed that as the number of prey increases, the number of prey consumed also increases [i.e., *C. mildei* (Araneae: Clubionidae) preying on different densities of *Spodoptera littoralis* (Boisduval) (Mansour et al. 1980a,b) and for *Philodromus rufus* (Araneae: Thomisidae) consuming various densities of densities of *Drosophila* (Haynes and Sisojevic 1966)]. Results of our feeding trial follow the same trend with that of the other species of spiders, which is a characteristic of various predators (Holling 1961, Huffaker et al. 1971). Nevertheless, our results should be verified by increasing the number of different prey densities given to a single spider.

Significantly more citrus leafminer larvae were consumed when two to three spiders were placed inside each cage (Fig. 3). There was a significant correlation [$r^2 = 0.987$; $y = 2.25 + 28.25\,(x)$] between the number of spiders and percent citrus leafminer consumption. It is evident from the results of this experiment that as the

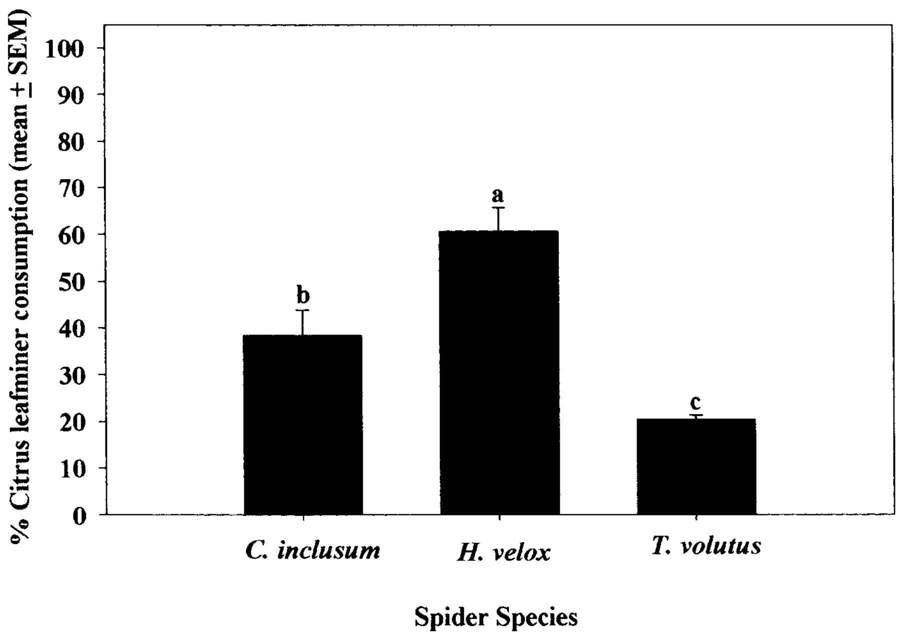

Fig. 1. Predation rate of *C. inclusum, H. velox,* and *T. volutus* on citrus leafminer larvae under greenhouse conditions. There were 20 citrus leafminer larvae in each trial. Bars with the same letters are not significantly different according to DMRT ($P \leq 0.05$).

Table 2. Average citrus leafminer larvae consumption by a single *Hibana velox* exposed to different densities of first- and second- instar larvae of citrus leafminer

Predator: prey ratio	Mean weekly prey consumed/predator ± SEM
1:1	1.0 ± 0.0 c
1:5	3.7 ± 0.6 c
1:10	5.3 ± 3.2 b
1:25	13.3 ± 2.3 a

Means ± SEM followed by the same letters are not significantly different ($P \leq 0.05$) according to DMRT.

number of spiders increased, the prey consumption also increased. The predator response in relation to prey density may be an adequate indicator of predatory potential. However, there is a possibility that the activity of the predator may be affected by predator density if predators interact (Burnett 1958).

All the results obtained from the different feeding efficiency tests confirmed our hypothesis that these hunting spiders could be one of the mortality factors of citrus

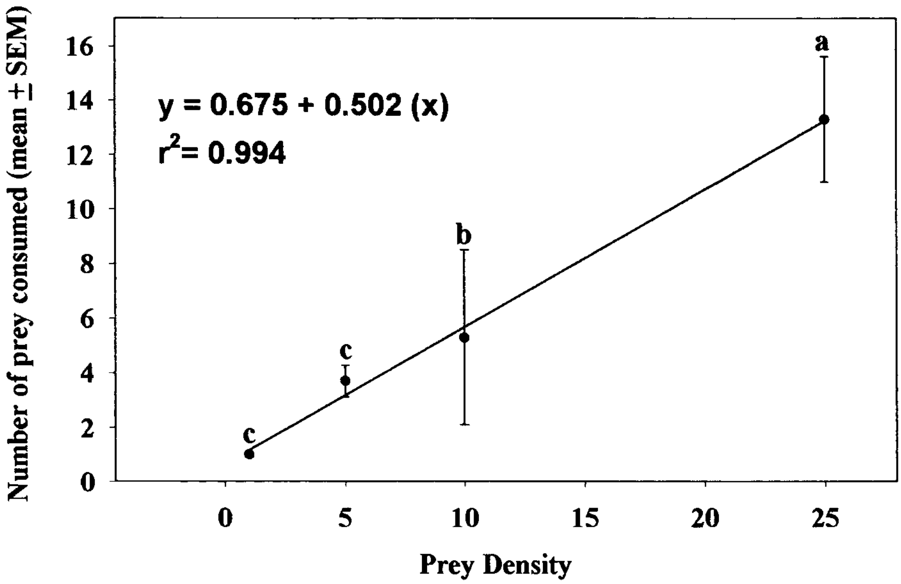

Fig. 2. Effect of population density of citrus leafminer larvae on the number of larvae consumed upon exposure to *H. velox*. Points with the same letters are not significantly different according to DMRT ($P \leq 0.05$).

leafminer in lime orchards. Previous feeding tests conducted on related species of hunting spiders on other lepidopterous pests had given similar results. For instance, Carroll (1980) reported that hunting spiders in the families Clubionidae and Anyphaenidae preyed on a number of important arthropod pests such as lepidopterans, mites, and thrips and concluded that hunting spiders contribute to control of lepidopterous pests in California citrus orchards. Corrigan and Bennett (1987) also reported that *Chiracanthium mildei* sought out apple trees infested with spotted tentiform leafminer, *Phyllonorycter blancardella* (Lepeletier). Other species of hunting spiders, particularly members of the anyphaenid family, were found feeding on and reducing numbers of the spotted tentiform leafminer larvae on apple trees. Although these hunting spiders in field crops are highly polyphagous, it has been shown that they can narrow their feeding niche significantly when a suitable prey species reaches high numbers (Nyffeler et al. 1994). It is, therefore, possible that the hunting spiders in lime orchards have the same predatory activity. Nevertheless, whether spiders are effective predators of citrus leafminer in lime orchards cannot be answered conclusively yet. More studies should be conducted in other geographical areas to further examine the efficiency of the spider species known to feed on citrus leafminer. The effect of the spider assemblage and their compatibility with other natural enemies should also be examined. For instance, field experiments combining the different spider species and the other natural enemies with known association on citrus leafminer (i.e., green lacewings, ants, and parasitoids) may elucidate the relative importance of each biotic mortality factor.

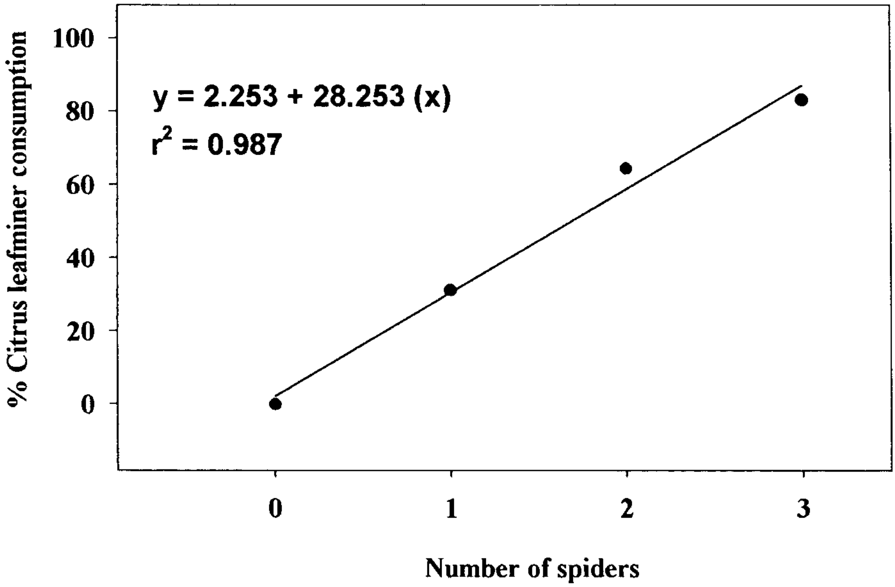

Fig. 3. Effect of different numbers of *H. velox* on the percent citrus leafminer consumption. There were 25 citrus leafminer larvae in each trial. Points with the same letters are not significantly different according to DMRT ($P \leq 0.05$).

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