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FECUNDITY, HOST STAGE PREFERENCES AND THE EFFECTS OF TEMPERATURE ON *PNIGALIO MINIO* (HYMENOPTERA: EULOPHIDAE), A PARASITOID OF *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE)

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Abstract. **The biology of the eulophid parasitoid *Pnigalio minio* (Walker) was studied in the laboratory and field in Florida on the gracillarid *Phyllocnistis citrella* Stainton. *P. minio* is a polyphagous ectoparasitoid of late instar larvae and prepupae. Oviposition in the field was highest (72.7%) on third instar *P. citrella*, with the remainder on the prepupa. Percent superparasitism in the field was 6.25 ± 4.35%. Offspring sex ratio from females provided with males was 64.9 ± 3.9% males and 35.1 ± 3.9% females. Laboratory studies showed that females provided with males began oviposition 3 to 6 days after emergence and continued ovipositing to 38.2 ± 6.5 days after emergence. Females laid a mean of 234 ± 58.9 eggs each, and female longevity reached 46.8 ± 9.2 days at 27 ±1°C, 80-90% RH. Longevity of males was lower, with a mean of 7.3 ± 0.6 days. Host feeding or stinging without oviposition killed 92.3 ± 16.6 *P. citrella* larvae per female. Unmated females produced only male progeny. Laboratory studies showed that *P. minio* development was shortest (8.6 ± 0.2 days) at 30°C and increased as temperature decreased (24.9 ± 0.4 days at 18°C). A brief description of the parasitoid egg, larva and pupa is provided.**

Phyllocnistis citrella Stainton is a leafminer that during larval stages tunnels under the cuticle destroying the epidermal cells, causing severe damage to several species of *Citrus* throughout the world (Clausen, 1931; Beattie, 1993; Heppner, 1993). Extensive mining causes the leaves to become distorted, and at times abscise. Consequently, there is a reduction of growth of young plants and a decrease in yield 1-2 years after initial injury (Mingdu and Shuxin, 1989; Hunsberger et al., 1996).

Diverse complexes of hymenopterous parasitoids are recognized as the most important natural enemies of *P. citrella* (Ding et al., 1989; Peña et al., 1996). Most parasitoids of *P. citrella* are eulophids; however, the complexes are usually complemented by encyrtids, elasmids, eurytomids and pteromalids (Ishii 1953). High levels of parasitism in south Florida were caused by species in 6 eulophid and elasmid genera during the past three years. The vast majority of these parasitoids (>80%) were *Pnigalio minio* (Walker) (Peña et al. 1996). *P. minio* (Walker) is a senior synonym of *P. proximus* (Ashmead) and *P. flavipes* (Ashmead) (Evans, 1999). *P. minio* is an ectoparasitic, polyphagous eulophid also found parasitizing *Liriomyza sativae* Blanchard on *Zizyphus mauritiana* and another dipterous leafminer on *Cassia* (R. E. Duncan, pers. obs.). *P. minio* is found throughout Florida and survives well in the southern coastal areas where overlapping leafminer generations provide susceptible stages for extended periods (Browning and Peña, 1995). Here we report laboratory and field studies on survival, fecundity, sex ratio, superparasitism, host instar preferences, mortality caused by adult wasps, and development time at different temperatures of *P. minio* on its host *P. citrella*.

Materials and Methods

Our *P. citrella* colony was initiated with pupae collected in the field in Homestead, FL and maintained in a greenhouse on 1 year-old *Citrus latifolia* trees following a methodology similar to that described by Smith and Hoy (1995). *P. minio* adults were obtained from field gathered material kept in lab cages until emergence. Lab experiments were conducted at 27± 1°C, 80-90% RH, 12:12 (L:D) unless otherwise noted. In experiments for which *P. minio* eggs were reared to adulthood, shoot segments (newly expanded leaves attached to a portion of the stem) with parasitized host larvae were placed in 10 ml glass vials filled with water. They were held in clear Plexiglas cages (30 × 30 × 30 cm) until the parasitoid pupa formed, then each leaf was transferred to individual Petri dishes (50 mm diam. × 9 mm) for emergence. Adult *P. minio* were maintained in similar cages and fed 50% apple juice solution. Mines examined for the presence of eggs on or near the host larvae were observed with a dissecting microscope. To accurately detect *P. minio* eggs, we first directed an overhead light beam on the host larva and then observed it with the understage lighting. When the quality of the leaf or the stage of the leafminer did not permit clear viewing, the mines were dissected.

Field Studies: Host instar preference, superparasitism and sex ratio. Material for determining the preferred instar and superparasitism was collected from a commercial *Citrus latifolia* orchard. Eighty-three *P. citrella* larvae of each instar were randomly selected and examined under the microscope to determine the presence of *P. minio* eggs. Host instar preference was determined by counting the number of molted head capsules in the mine. Parasitoid eggs were left undisturbed and reared to adults to verify species.

An additional set of *P. minio* eggs (n = 154) were collected from the field and reared to adulthood to ascertain population sex ratio.

Laboratory Studies: Fecundity and Longevity. P. minio females were individually placed in cages within a day of emergence, only females that lived long enough to start ovipositing were used (n = 6). Each female was provided 2 males, and from 3 to 37 second and third instar *P. citrella* larvae. The number of host larvae offered varied according to the fecundity of the parasitoid. Mating was assumed to occur. The fate of the ex-

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posed larvae and the parasitoids was recorded daily and a new set of host larvae re-introduced into the cage. Exposed larvae were defined as parasitized if one or more eggs were deposited on or near the host. Female *P. minio* were maintained for their entire life and males were replaced as they died. To determine F_1 fecundity *P. minio* daughters (n = 3) were individually placed in a cage with males following the same methodology as explained above, and held until we had collected about 108 eggs per female. F_2 individuals were reared to adulthood.

Host mortality due to host-feeding and stinging. Evidence of stinging and/or feeding on citrus leafminer larvae ranged from no detectable markings or a very small black dot left after insertion of the ovipositor, to partial or complete desiccation due to feeding by the adult female parasitoid on the haemolymph. Host mortality in the absence of *P. minio* was determined by placing infested leaves with unparasitized *P. citrella* larvae (n = 100) in a cage with no parasitoids. Larval survival was observed daily and a new set of host larvae was introduced into the cage.

Virgin females. To determine sex ratio of the progeny of unmated females, one week old virgin females (n = 5) were caged individually, in the absence of males, with host larvae and the fate of the hosts and sex ratio of the *P. minio* progeny recorded. Virgin females were held until we had collected about 16 eggs from each.

Male longevity. Individual *P. minio* males (n = 18) less than 1 day old were confined in clear Plexiglas cages and provided with apple juice as explained above. Their survival was observed daily. Host material and a female were also provided to simulate a more natural environment.

Immature Stages. Ectoparasitoid eggs were collected in the field and reared to adulthood to verify species. The parasitoids were observed and measured as they developed. Eggs (n = 15) were measured at 50×, larvae (n = 14) and pupae (n = 15) were measured at 25×.

Development time at various temperatures. Eggs were obtained for this experiment from a series of cages containing four to six one-week old *P. minio* females, with approximately twice as many males, provided with unparasitized second and third instar hosts. Oviposition was recorded and the position of *P. minio* eggs in relation to the host larva noted by marking the leaf and making a diagram. Twenty to 33 parasitized *P. citrella* larvae per temperature were held in environmental chambers at 18, 21, 24, 27 and 30°C, 80-90% RH, 12:12 (L:D). Parasitoid development was observed daily until emergence.

Statistical analysis. The data were subjected to analysis of variance followed by Waller-Duncan multiple comparisons, using SAS (SAS Institute 1987). Data are expressed as mean ± SEM.

Results and Discussion

Field Studies: host instar preference, superparasitism and sex ratio. Host instar significantly affected the number of eggs oviposited by a *Pnigalio minio* female under field conditions (Table 1). Eggs were found mostly on third instars (72.7%), with the rest on the prepupal stage (27.3%), showing a significant preference for third instars. Eggs oviposited near a late third instar or prepupa would occasionally become trapped in the silk of the cocoon as the host larva spun its pupal case. Under laboratory conditions females rarely oviposited on the second instar and pupal stage.

Table 1. The percent of *Pnigalio minio* eggs found per instar on its host larva, *Phyllocnistis citrella* (n = 83 per instar), in the field.

Host instar	Percent of parasitoid eggs oviposited per instar
First	0.0 c
Second	0.0 c
Third	72.7 a
Prepupa	27.3 b
Pupa	0.0 c

Means followed by the same letter are not significantly different (P > 0.0001; df = 411; F = 28.52; Waller-Duncan multiple comparisons).

Superparasitism on third instar hosts was 6.25 ± 4.35% (n = 83) under field conditions. No superparasitism was noted on the prepupae. When confined in a laboratory cage, however, females oviposited up to six eggs per host larva. The adults usually did not harm eggs previously oviposited on or near the host larva. But upon eclosion the parasitoid larva searched the mine area surrounding the host larva and killed any other parasitoid eggs it found.

The sex ratio of *P. minio* eggs collected from the field was 64.9 ± 3.9% males and 35.1 ± 3.9% females (n = 154).

Laboratory studies: fecundity and longevity. The presumably mated females began ovipositing 3-6 days after emergence and continued ovipositing up to day 38.2 ± 6.5 (Fig. 1A). The number of days each female oviposited was 14, 28, 38, 40, 50, and 59. The ovipositing female inserted its ovipositor into or near the host three or four times, about 10 seconds each time, to sting and oviposit. The egg was deposited on or within 1 mm of the host larva. The peaks of age-specific fecundity were 8.0 ± 3.0 and 8.5 ± 3.9 eggs per female per day, observed on 13 and 34 day-old *P. minio* females respectively. Females each oviposited a total of 234.0 ± 58.9 eggs in their lifetime and lived 46.8 ± 9.2 days. Females (n = 3) from the F_1 generation successfully mated and produced an F_2 (mean n = 55). Host larval mortality from oviposition was 165.7 ± 38.2 per female, ranging from 69 to 277.

Host mortality due to host-feeding and stinging. Host killing by adult females without parasitism began simultaneously with oviposition and ended when the parasitoids ceased ovipositing. *P. minio* females exhibited their highest host-killing peak approximately 15 days after emergence, killing 3.7 host larvae per day per female (Fig. 1B). To feed, female adults would alternately puncture and rasp the host larvae with their ovipositors, twisting the inserted ovipositor from side to side, withdrawing it and then feeding for approximately 20 seconds on the exuding haemolymph. This behavior was observed as many as 9 times on a single host larva.

Females preferred to sting or feed on second and early third instar larvae. Hosts killed by parasitoid feeding or stinging without oviposition reached 92.3 ± 16.6 per female. This feeding and stinging accounts for 35.8% of the mortality caused by an adult female, a factor that should be noted when attempting to measure mortality caused by this parasitoid in the field. Previous researchers have noted the importance of host feeding and mechanical injury when evaluating mortality caused by parasitoids (DeBach 1943, Van Driesche et al. 1987). In studies of *P. minio* on *Phyllonorycter elmaella* Doganlar and Mutuura, 32.7% of mortality was caused by host feeding, and 10.1% by host stinging without oviposition (Barrett and Brunner 1990, as *Pnigalio flavipes*). In the case of the *P. citrella*, *P. minio* feeding on second and third instars may be important, since a large portion of the damage and reduction of leaf

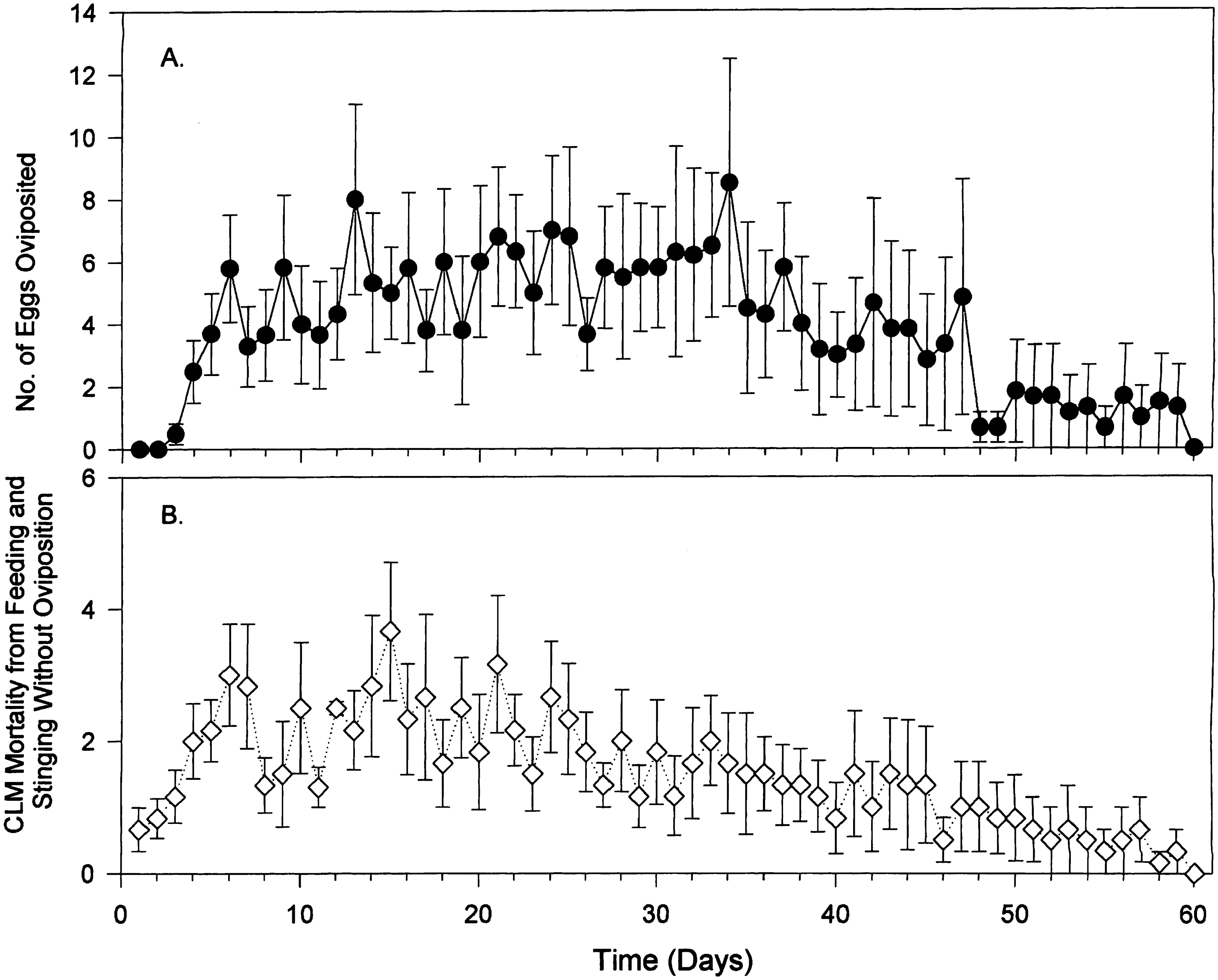

Figure 1. Daily oviposition and mortality caused by *Pnigalio minio* on its host larvae *Phyllocnistis citrella* (n = 6; mean ± SEM). (A) Mean number of eggs oviposited per female. (B) Mean larval mortality from host feeding and stinging without oviposition per female.

area is caused by the third instar (B. Schaffer, Univ. of Fla., Institute of Food and Agricultural Science, unpublished data).

Almost 100% of the citrus leafminer larvae kept without parasitoids as a control measure survived. Thus, mortality caused by experimental handling in the above experiment is a negligible factor (ca. > 0.001%). In summary, total cumulative mortality from successful parasitism, feeding, and stinging without oviposition, reached 258.0 ± 54.1 (ranging from 116 to 489) host larvae per female in her lifetime.

Virgin females. P. minio offspring from virgin females (n = 5) resulted in production of only male progeny. Of the 16 eggs collected per female a mean of 10.4 developed to adulthood. In contrast females provided with males produced both male and female progeny (43.4% females; n = 465) confirming *P. minio* arrhenotoky.

Male longevity. Males lived an average of 7.3 ± 0.6 days (n = 18) after emergence.

Description of P. minio immature stages. The egg is elongated, smooth, and milky white. It is 0.314 ± 0.004 mm long, 0.082 ± 0.001 mm diameter at the smaller end and 0.111 ± 0.002 mm diameter at the wider end. As the egg matures, it becomes partially and then totally translucent. The larva is flattened and colored translucent beige, with the alimentary canal content visible. This content is at first white or beige and turns darker brown as the larva matures. The larvae reach 1.87 ± 0.08 mm long and 0.52 ± 0.03 mm wide when they stop feeding. At lower temperatures the parasitoid larva will sometimes migrate (approx. 6-10 mm) and pupate away from the host larva. At higher temperatures, pupation usually takes place near the host. Just before the larva pupates, it becomes cylindrical and exudes a meconium, turning milky white. The pupa is milky white at first but soon turns dark brown to black, reaching 1.49 ± 0.07 mm in length for the male pupa and 1.76 ± 0.05 mm for the female. The width of the pupal head is 0.49 ± 0.02 mm, 0.58 ± 0.03 mm for the thorax and 0.49 ± 0.02 mm for the abdomen.

Development time at various temperatures. Development time decreased in all stages as the temperature increased (Table 2).

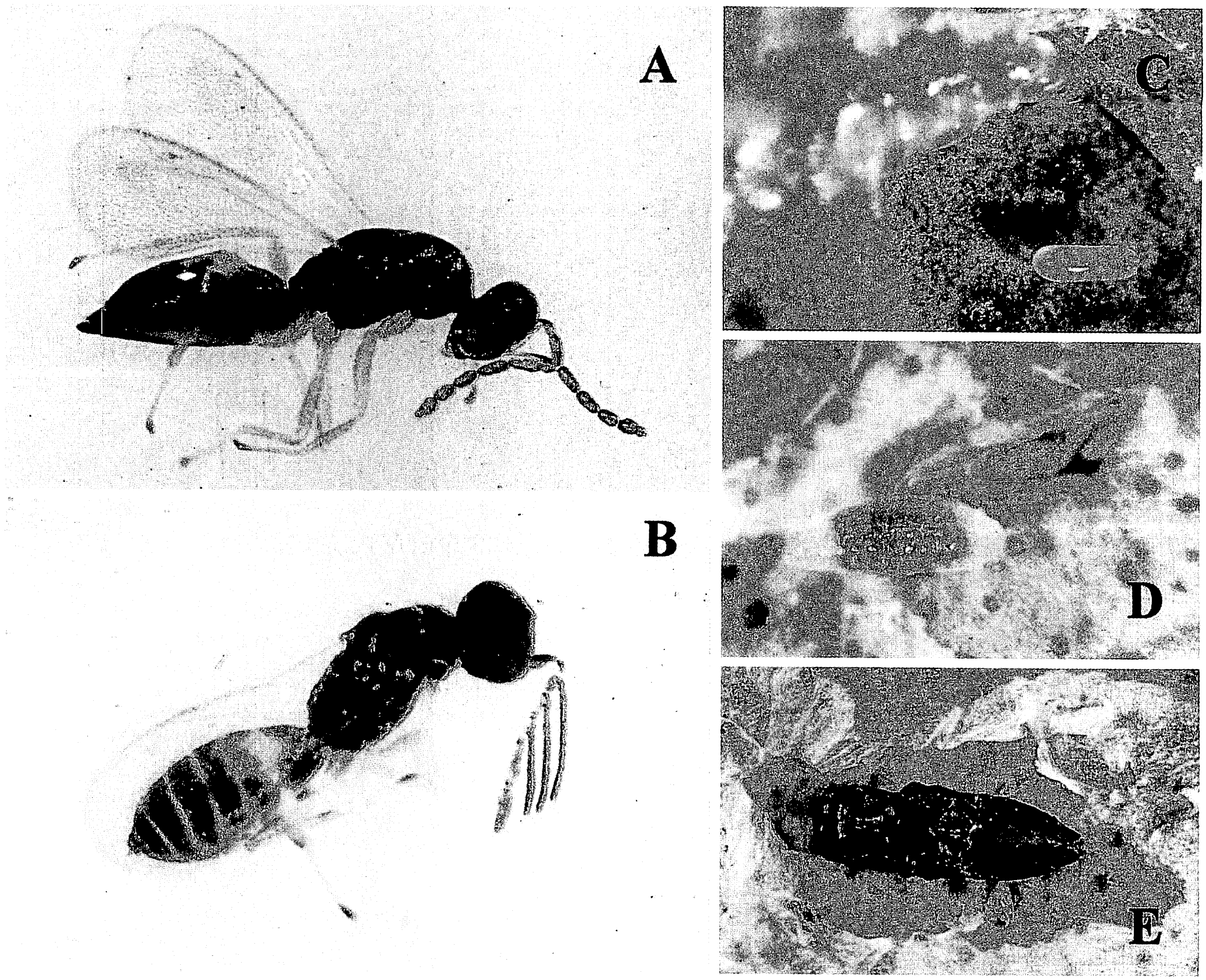

Figure 2. *Pnigalio minio* (Walker). (A) Female. (B) Male. (C) Egg. (D) Larva. (E) Pupa.

Mortality was not significantly different (P = 0.6115) at the temperatures used in this experiment. This suggests that development could successfully occur at higher and lower temperatures than those tested, and that it may therefore be useful as a biological control agent over a wide range of habitats and seasons.

Table 2. Development time of *Pnigalio minio* at various temperatures on *Phyllocnistis citrella* (mean ± SEM).

Temp. (°C)	Days from Egg to larva (n)	Days from larva to pupa (n)	Days from pupa to adult (n)	Days from egg to adult (n)
18	2.5 ± 0.1 (18)	9.0 ± 0.4 (18)	13.5 ± 0.4 (26)	24.9 ± 0.4 (26)
21	1.2 ± 0.1 (13)	6.3 ± 0.2 (28)	8.6 ± 0.2 (28)	16.1 ± 0.2 (28)
24	1.3 ± 0.2 (9)	4.2 ± 0.1 (15)	6.3 ± 0.2 (15)	11.9 ± 0.2 (15)
27	1.0 ± 0 (14)	3.5 ± 0.1 (12)	5.8 ± 0.1 (15)	10.3 ± 0.2 (15)
30	1.0 ± 0 (24)	3.1 ± 0.1 (28)	4.4 ± 0.2 (29)	8.6 ± 0.2 (29)

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A SURVEY OF BLUEBERRY ACREAGE IN FLORIDA

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Abstract. **During spring, 2000, a survey form was mailed to all county extension offices in Florida where commercial blueberries are grown. Survey participants were asked to report the number of commercial blueberry acres in their counties and categorize acreage by type (southern highbush or rabbiteye) and by age (<3 years old, or 3 years old or older). Results of the survey indicated that total statewide blueberry acreage decreased between 1989 (the date of a previous survey) and 2000. However, southern highbush (SHB) acreage had increased by about 23%. The region of greatest decline in blueberry acreage was in the Florida panhandle west of the Apalachicola River which contained primarily rabbiteye (RE) blueberry farms. The region of greatest expansion was central peninsular Florida where SHB blueberries grown for the commercial shipping market predominate. The leading blueberry-producing counties in Florida in terms of acreage in descending order are; Alachua, Highlands, Polk, Hillsborough, and Lake. Approximately one-third of all blueberry acreage in the state was less than 3 years old. Commercial SHB acreage harvested for the fresh fruit shipping market is increasing in Florida, but the acreage of rabbiteye for local sales appears to be less than the market could sustain, especially in areas near large population centers.**

Florida is the major producer of fresh blueberries between 1 April and 20 May. Fruit prices are high during this period due to a large demand for fresh blueberries coupled with a limited supply. The most recent detailed survey of blueberry acreage in Florida was conducted in 1989 by Crocker and Willis. They reported a total acreage of approximately 2100. This represented an increase of almost 100% during the 5-year period between 1989 and 1984 when an earlier survey was conducted (Crocker and Lyrene, 1985). More recently, significant changes in Florida's blueberry industry have occurred, including establishment of many new plantings of southern highbush (SHB) cultivars and decreased rabbiteye (RE) production. The purpose of this survey is to determine the current acreage of commercial blueberries in Florida by, type (SHB vs RE), age, and location in the state.

Materials and Methods

During spring, 2000, a survey questionaire was mailed to all county extension offices in Florida counties where blueberries were grown commercially. County extension faculty in each county were asked to provide information on the total number of commercial SHB and RE blueberry acres in their counties, the number of acres that were less than three years old, the number of blueberry farms, and whether the majority of fruit from each farm was handled through commercial shipping markets, or sold locally (u-pick, roadside, etc.). Information was obtained by phone for counties that did not respond to the original mailing. All counties where commercial blueberries are grown were represented. For the purposes of reporting data, Florida was divided into four production regions: 1) northwest (counties in the panhandle west of the Apalachicola River; 2) north (counties east of the Apalachicola River and north of Citrus, Sumnter, Lake and Volusia counties); 3) central (counties south of the northern region and north of Manatee, Hardee, Okeechobee and Indian River counties); and 4) south (counties south of the central region).

Results and Discussion

Table 1 shows RE and SHB blueberry acreage by region. The northern region contained the largest total acreage of blueberries and the largest acreage of each type. The central region ranked second in total acreage and second in SHB acreage. Total blueberry acreage decreased for the western and northern regions since 1989. This is primarily because

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