Impacts of Parasitism on the Survivorship and Reproduction of the Madeira Mealybug (Hemiptera: Pseudococcidae)[1]

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Abstract This study was conducted to assess the impacts of parasitism by *Anagyrus* sp. nov. nr. *sinope* Noyes and Menezes on the reproductive potential and the survivorship of the Madeira mealybug, *Phenacoccus madeirensis* Green. The reproductive responses of *P. madeirensis* to parasitism were dependent upon the reproductive status of the adult mealybugs. The reproductive period and fecundity of parasitized preovipositing adult mealybugs were significantly lower (4 d and 290 eggs, respectively) than those of the unparasitized individuals (8 d and 380 eggs, respectively). However, the reproductive longevity and fecundity were not different between the parasitized and unparasitized ovipositing mealybugs. Destructive feeding by parasitoid larvae reduced the total longevity of all adult mealybugs by 1-4 d. The parasitoid only host fed on 2.4% of the 1st-instar mealybugs and none in other developmental stages. In the 4 developmental stages examined (1st, 2nd and 3rd nymphal instars, and prereproductive adult), only the survival rate of 1st-instar nymphs was significantly reduced by ovipositor insertion (97% of the unparasitized versus 88% of the parasitized). After accounting for the mortality due to host feeding and ovipositor insertion, the parasitism rate of *P. madeirensis* by *Anagyrus* sp. nov. nr. *sinope* was estimated at 30%.

Key Words fecundity, host feeding, host stage selection, longevity, ovipositor insertion

The parasitoid *Anagyrus* sp. nov. nr. *sinope* Noyes and Menezes (Hymenoptera: Encyrtidae) is a potential biological control agent of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), a serious pest of horticultural and agronomic crops worldwide. Chong and Oetting (2006a, 2007) studied the host stage selection behavior of *Anagyrus* sp. nov. nr. *sinope* and concluded that the 3rd-instar immature and prereproductive adult female *P. madeirensis* were the most preferred host stages for parasitism and development. *Anagyrus* sp. nov. nr. *sinope* is a koinobiont parasitoid, with 1, 23, and 75% of the 1st-, 2nd-, and 3rd-instar nymphs, respectively, able to develop into adults after parasitization (Chong and Oetting 2006a). Half of the prereproductive adult mealybugs and all of the ovipositing mealybugs continued to reproduce after parasitization. However, Chong and Oetting (2006a) did not investigate the difference in survivorship and reproductive patterns between the parasitized and the unparasitized adult mealybugs.

Continuous reproduction of parasitized hosts has significant impacts on the population dynamics of the hosts and the parasitoids. The ability of the parasitized mealybugs to reproduce may reduce the suppression of mealybug population by the

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parasitoid (Lin and Ives 2003). On the other hand, model simulations have suggested that the continuous production of a low number of eggs, which are invulnerable to parasitism and contribute to maintenance of the population, by the parasitized hosts may stabilize host-parasitoid population interactions (Spataro and Bernstein 2000).

Parasitoids cause mortality in a host population in three ways: parasitization, injury, and host feeding (Van Driesche et al. 1987). *Anagyrus* sp. nov. nr. *sinope* parasitized between 17-40% of *P. madeirensis* depending on the ambient temperature (Chong and Oetting 2006b). Host feeding has been occasionally observed in *Anagyrus* sp. nov. nr. *sinope* (Chong and Oetting 2007) and is common in other parasitoid species (Jervis and Kidd 1986, Heimpel and Collier 1996). The extent to which *P. madeirensis* died of mechanical injury caused by ovipositor insertion is unknown.

This study was designed to investigate the impact of parasitism on the reproduction, and the importance of host feeding and ovipositor insertion as mortality factors, of *P. madeirensis*. The results will reveal the impacts of parasitism on the population dynamics of *P. madeirensis* and the mortality rate of *P. madeirensis* after considering mortality due to host feeding and ovipositor insertion.

Materials and Methods

The first experiment assessed the adult longevity, reproductive period, and fecundity of *P. madeirensis* after parasitization. Thirty young prereproductive adult mealybugs (2-3 mm) and 30 mature adult mealybugs (3-4 mm, began to produce wax filaments for ovisac construction but not yet produced eggs) were collected from an insectary colony of P. *madeirensis* maintained on sprouted potatoes (*Solanum tuberosum* L.). The mealybug colony was maintained in an insectary, and the parasitoid colony was maintained in a greenhouse, at the University of Georgia Griffin Campus (Griffin, GA). The mealybugs within each size group were divided between 2 treatments: 'parasitized' (n = 20) and 'unparasitized' (n = 20). The mealybugs were isolated individually on an excised chrysanthemum (*Dendranthema* × *grandiflora* Kitam.) leaf in a Petri dish (15 × 100 mm). The excised chrysanthemum leaves were kept vigorous by inserting each petiole into a cup of water through a hole drilled on the bottom of each Petri dish. All the Petri dishes were kept in an environmental chamber maintained at 25°C, 90% R.H., and 14:10 (L:D) h photoperiod. The mature female mealybugs were allowed to reproduce for 2 d before parasitism; thus, given rise to a group of ovipositing adult mealybugs.

In the 'parasitized' treatment, an *Anagyrus* sp. nov. nr. *sinope* collected from the laboratory colony at Griffin Campus (Griffin, GA) was released into each Petri dish. The oviposition behavior of the parasitoid was observed, and the parasitoid was removed after the mealybug had been parasitized (evidenced by the prolonged ovipositor insertion; Chong and Oetting 2007). No parasitoid was released into the Petri dishes that were assigned to the 'unparasitized' treatment. The mealybugs in both 'parasitized' and 'unparasitized' treatments were kept in the environmental chamber and allowed to lay eggs until death. The mealybug eggs were collected and counted every 24 h. The duration from parasitization to death (adult longevity) and the duration of reproduction (reproductive period) also were recorded for each mealybug.

Mealybugs of the 'parasitized' treatment that did not mummify or died before mummification, and those of the 'unparasitized' treatment that died before reproduction, were excluded from the statistical analysis. The data were analyzed with a 2-way

factorial analysis of variance (ANOVA) with the host stage and parasitization status as the main factors. The means were separated by Tukey's honestly significant difference (HSD) test when significant difference among the means was detected at a threshold of 0.05 (SAS Institute 1999).

The second experiment assessed the mortality rate of various developmental stages of *P. madeirensis* within a 4-d period after parasitization. Preliminary observations suggested that host-fed or severely injured mealybugs usually died, and the parasitized mealybugs were not mummified, earlier than 4 d after parasitization. To standardize mealybug age and minimize mortality due to transfer, the mealybug cohorts were initiated with eggs collected from the insectary colony within 24 h. The eggs hatched and the nymphs were reared on excised chrysanthemum leaves in Petri dishes at 25°C, 90% R.H., and 14:10 (L:D) h photoperiod. The nymphs were reared to 1st instar (3 d after egg eclosion), 2nd instar (12 d), 3rd female instar (20 d), and prereproductive adult (26 d). In each host stage, the Petri dishes were divided between 2 treatments: 'parasitized' (n = 20) and 'unparasitized' (n = 20). A female parasitoid was released into each Petri dish, and its foraging behavior was observed for host feeding and ovipositor insertion. The parasitoid was removed after 5 or 6 of the mealybugs in each Petri dish had been probed. The mealybugs that were not probed were removed from the 'parasitized' cohorts. A similar number of mealybugs were kept in the Petri dishes assigned to the 'unparasitized' treatment. The mealybugs were incubated in the environmental chamber for 4 d, and the mortality rate was determined by dividing the number of mealybugs that died over the 4-d period with the initial number of mealybugs. The numbers of escaped and host-fed mealybugs were not included in the calculation of mortality rate. The mortality rate was arcsine-transformed before subjected to analyses with the 2-way ANOVA and separated with the Tukey's HSD test (SAS Institute 1999).

Results and Discussions

Reproduction after parasitization. All parasitized prereproductive and ovipositing mealybugs mummified in 6-7 d after parasitism. Within each parasitism status, the duration from parasitization to death and the reproductive period were not different between prereproductive or ovipositing *P. madeirensis* (Figs. 1 and 2). Ovipositing females produced slightly more eggs than the prereproductive females of the same parasitism status (Fig. 3). For both the prereproductive and the ovipositing mealybugs, the parasitized mealybugs died earlier than the unparasitized individuals (Fig. 1). The unparasitized prereproductive and ovipositing mealybugs lived for 10 and 8 d, whereas the parasitized ones for only 6 and 7 d, respectively. The unparasitized prereproductive mealybugs continued their development and reproduced for 4 d longer (Fig. 2) and 100 (or 32%) additional eggs (Fig. 3) than the parasitized prereproductive mealybugs. The reproductive longevity and total fecundity were similar between the parasitized and unparasitized ovipositing mealybugs (Figs. 2 and 3).

The results suggested that the effect of parasitism on the reproduction of *P. madeirensis* was dependent upon the reproductive status of the mealybugs. If the adult females were parasitized before oviposition began, the females lived for a shorter time before death and reproduced for a shorter duration and produced a fewer number of eggs. Once the mealybugs were committed to reproduction, parasitism did not reduce the reproductive longevity and the fecundity, but the parasitized mealybugs died earlier than the unparasitized mealybugs.

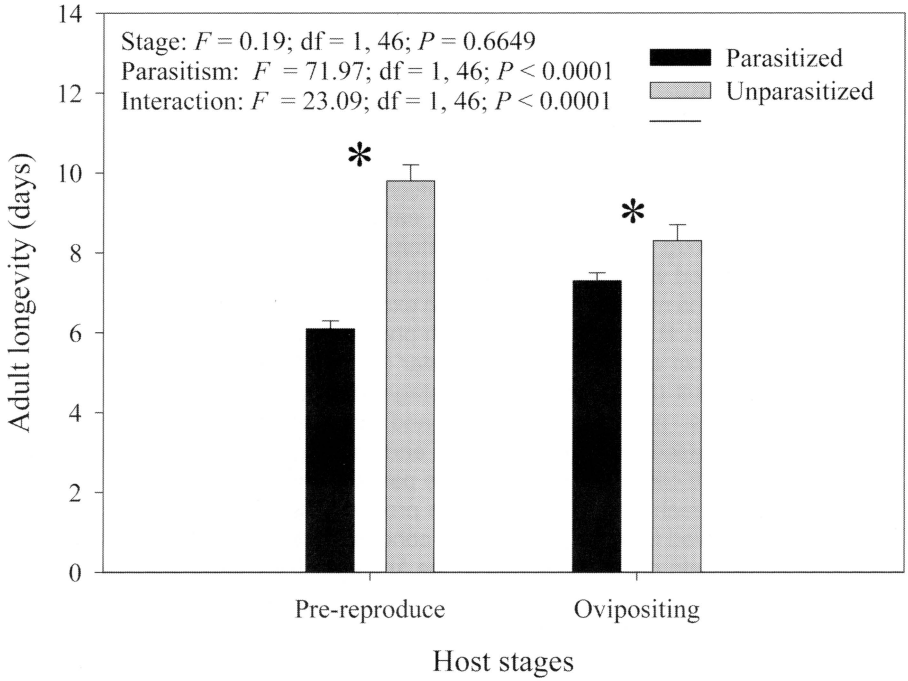

Fig. 1. Duration from parasitization to death (or adult longevity) of parasitized and unparasitized adult *P. madeirensis* of the two reproductive status (prereproductive and ovipositing). An asterisk denotes significant difference between parasitized and unparasitized mealybugs of the same reproductive status.

Citrus mealybug, *Planococcus citri* (Risso), parasitized by *Anagyrus pseudococci* (Girault) also ceased normal reproduction and produced a fewer number of eggs (Islam et al. 1997). Similar studies on several aphid species also showed that the adult longevity, reproductive period, and fecundity decreased in parasitized aphids but the reduction was smaller in older aphids than in younger aphids (Kairo and Murphy 1999, Tang and Yokomi 1996, Tsai and Wang 2002). The shorter lifespan of parasitized hosts between parasitization and death may be attributed to the destructive feeding and mummification by the parasitoid larvae within the hosts. The reduction in fecundity and reproductive longevity of parasitized mealybugs may be caused by either a competition for nutrients between the reproductive organs and the endoparasitic larvae (Godfray 1994) or the destruction and shrinkage of reproductive organs (Higaki 2003).

The ecological impact of continuous reproduction after parasitism is rarely examined. Instead, most population dynamics models consider the parasitized hosts as dead (Briggs and Godfray 1995). Reproduction rate is an important factor in determining the population dynamics of the hosts and the efficiency of the parasitoids (Spataro and Bernstein 2000). The production of a low number of eggs by parasitized hosts has the effect of stabilizing the host-parasitoid population dynamics. However, when the parasitized hosts continue to enjoy a high reproductive rate, the regulation

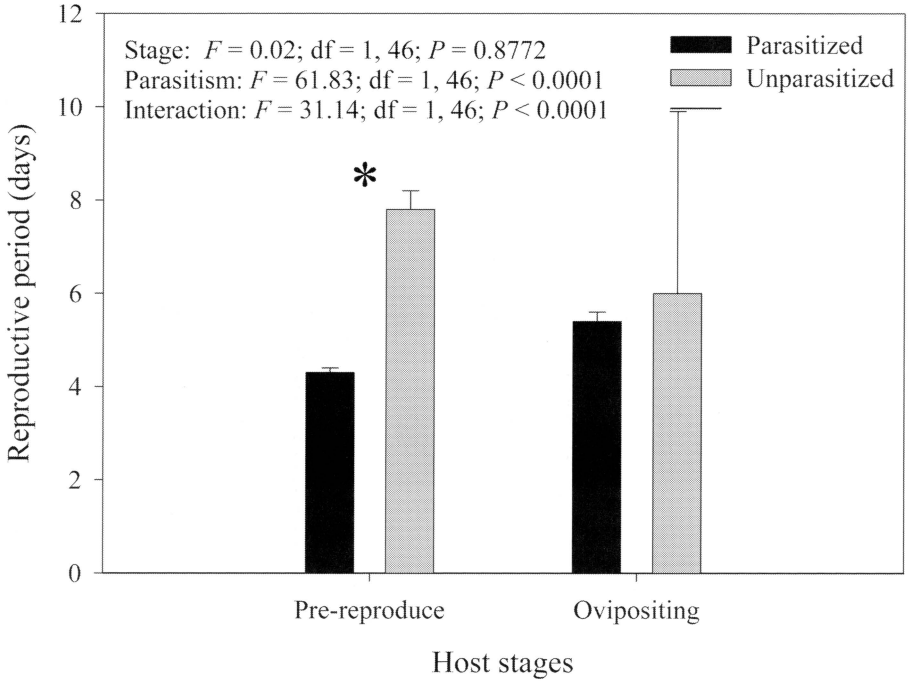

Fig. 2. Duration from the beginning to the end of reproduction (or reproductive longevity) of parasitized and unparasitized adult *P. madeirensis* of the two reproductive status (prereproductive and ovipositing). An asterisk denotes significant difference between parasitized and unparasitized mealybugs of the same reproductive status.

of host population by the parasitoids diminished. This study suggested that the regulation of *P. madeirensis* population by *Anagyrus* sp. nov. nr. *sinope* may be more effective when the parasitoids attack the younger adult *P. madeirensis*, which produced a fewer number of eggs after parasitization, than the more mature hosts whose fecundity are not affected by parasitism.

Host feeding and ovipositor insertion as mortality factors. Host feeding provides essential nutrients and proteins for egg production and maturation of the parasitoids (Jervis and Kidd 1986, Heimpel and Collier 1996). Only 2.4% of the 1st-instar *P. madeirensis* were host-fed by *Anagyrus* sp. nov. nr. *sinope* in this study. No host feeding was observed in parasitoids attacking the older mealybugs. These host-fed mealybugs appeared shriveled and died within 24 h of attack. *Anagyrus* sp. nov. nr. *sinope* used in this study were mature individuals collected directly from the laboratory colony. These parasitoids might have already satisfied their needs for host protein through host feeding in the laboratory colony; thus, resulting in a low incidence of host feeding in this study.

Host feeding by encyrtid parasitoids of mealybugs is documented in only a few cases because of a lack of detailed observations. Host feeding is often against mealybugs of the 1st and 2nd nymphal instars (Bokonon-Ganta et al. 1995). About 7 and

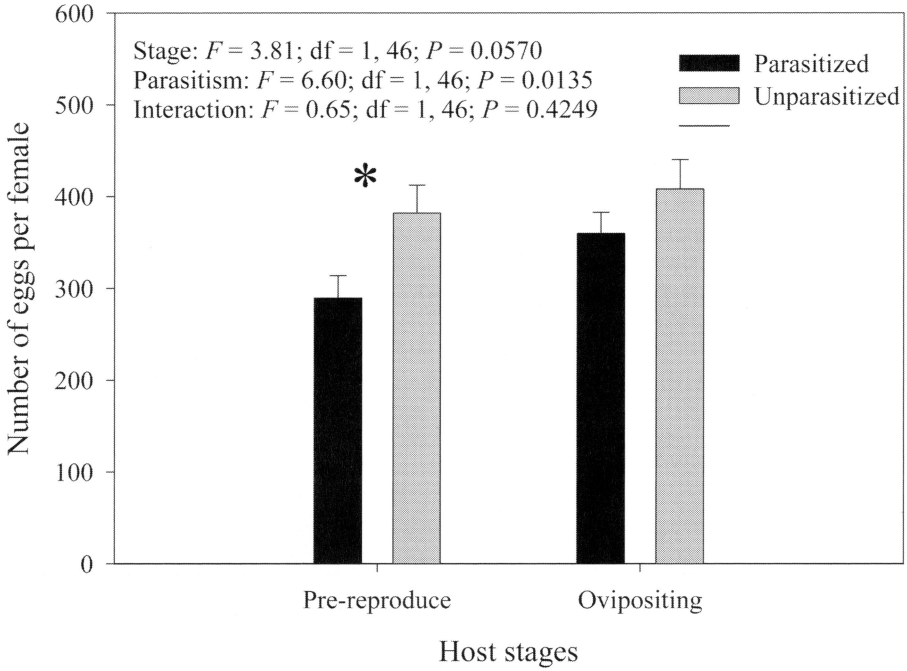

Fig. 3. Fecundity of parasitized and unparasitized adult *P. madeirensis* of the two reproductive status (prereproductive and ovipositing). An asterisk denotes significant difference between parasitized and unparasitized mealybugs of the same reproductive status.

4% of the 1st- and 2nd-instar nymphs, respectively, of the mango mealybug, *Rastrococcus invadens* Williams, were host-fed by *Anagyrus mangicola* Noyes (Bokonon-Ganta et al. 1995). *Gyranusoidea tebygi* Noyes fed on 12% of the mango mealybug (Boavida et al. 1995) and *Anagyrus* (= *Epidinocarsis*) *diversicornis* (Howard) fed on 9% of the cassava mealybug, *Phenacoccus herreni* Cox and Williams (Van Driesche et al. 1987). However, the percentages were not determined for individual host stage in the above studies.

When comparing among all developmental stages, mortality rate due to ovipositor insertion was significantly higher in the 1st- and 2nd-instar nymphs, with 12-14% of the individuals attacked died of the injuries within 4 d (Fig. 4). Ovipositor insertion only caused significant mortality in the attacked individuals of the 1st nymphal instar when compared with those that were not attacked (Fig. 4). More than 12% of the probed 1st-instar nymphs died of the injuries as compared with only 3% of the unparasitized nymphs that had died of other causes.

Mortality caused by factors other than parasitism has been difficult to quantify in the field because of the lack of physical evidence, e.g., mummies or persistent cadavers. Nevertheless, mortality caused by mechanical injuries and host feeding are important and integral parts of a comprehensive assessment of the performance of a biological control agent. For example, although the parasitism rate of the cassava

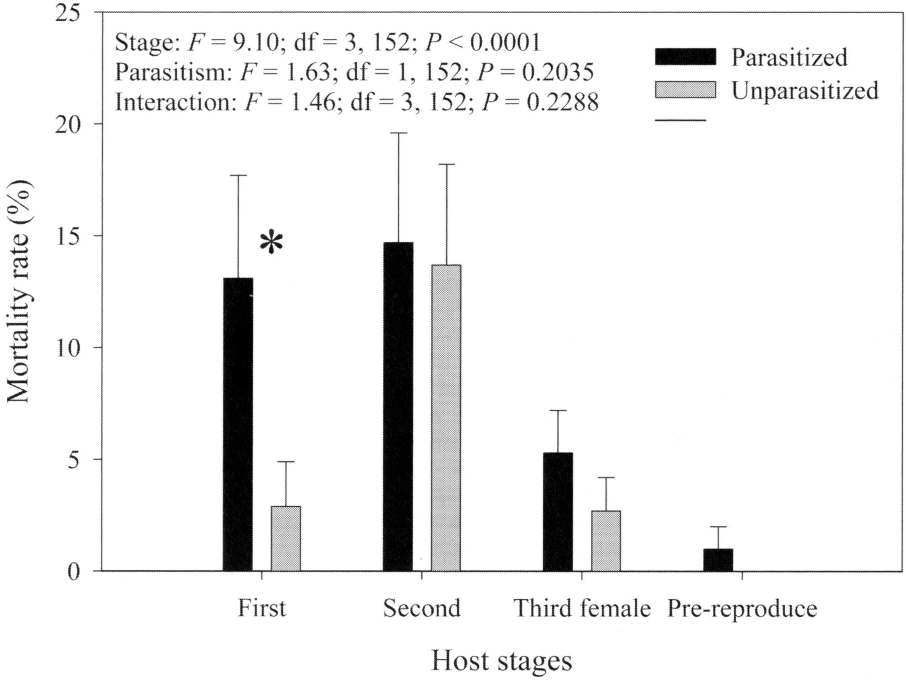

Fig. 4. Mortality rate of parasitized and unparasitized *P. madeirensis* of various developmental stages. An asterisk denotes significant difference between parasitized and unparasitized mealybugs of the same developmental stage.

mealybug, *Phenacoccus manihoti* Matile-Ferrero, by the parasitoid *Anagyrus* (= *Epidinocarsis* or *Apoanagyrus*) *lopezi* (DeSantis) was about 30% in the field, the additional mortality caused by host feeding helps to maintain an effective and sustainable biological control program in the field (Neuenschwander and Madojemu 1986).

Van Driesche et al. (1987) presented a formula to correct the estimate of total mortality: $y = x(1 - b)^{-1}(1 - a)^{-1}$, where the parameter y is the corrected estimate, x is the uncorrected mortality, and a and b are the mortality by host feeding and ovipositor insertion, respectively. At 25°C, a single *Anagyrus* sp. nov. nr. *sinope* is capable of parasitizing 28% of *P. madeirensis* (Chong and Oetting 2006b). With an average host feeding rate of 0.6% and an average mechanical injury rate of 7%, the corrected estimate of total mortality of *P. madeirensis* by *Anagyrus* sp. nov. nr. *sinope* is 30%.

In summary, the reproduction of *P. madeirensis* parasitized as prereproductive adults was more adversely affected by parasitism than that of the ovipositing females. Host feeding and mechanical injuries are significant mortality factors, especially in 1st- and 2nd-instar nymphs. These mortality rates should be considered when assessing the performance of the parasitoids in the field.

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