Research Note

HOST INSTAR PREFERENCE OF MIRAX INSULARIS *(MUESEBECK) (HYMENOPTERA: BRACONIDAE), A KOINOBIONT PARASITOID OF* LEUCOPTERA COFFEELLA *GUERIN-MÉNÉVILLE (LEPIDOPTERA: LYONETIIDAE)*[1,2]

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The coffee leafminer, *Leucoptera coffeella* (Guerin-Ménéville), is one of the main pests of coffee, *Coffea arabica* (L.), in Puerto Rico. Coffee leafminer larvae are parasitized by several eulophid species and one braconid, *Mirax insularis* Muesebeck (Seín, 1940; Wolcott, 1947; Gallardo, 1988). This braconid was introduced in Puerto Rico from Guadeloupe in 1937 (Seín, 1940). *Mirax insularis* is well distributed throughout all the ecological areas of the coffee region of Puerto Rico and exerts 14.8% parasitism on *L. coffeella* (Gallardo, 1988; 2006). This braconid is a koinobiont (larva-pupa endoparasitoid) and its life cycle has a duration of 15 to 17 days (Leon, 1997), which is very similar to that of *L. coffeella*.

Mass rearing and field liberations of *M. insularis* in an augmentation program to control the coffee leafminer have been addressed by Gallardo (1992). The fact that *M. insularis* is well established throughout the coffee region eliminates all the costly and time consuming steps (e.g., foreign explorations and quarantine procedures) needed when the classical or new association of biological control approaches are implemented. To establish such a program, mass production of the parasitoid and further releases at strategic times on selected coffee plantations in Puerto Rico are indispensable. For increasing chances of success in the mass rearing, information on sex ratio of *M. insularis* from field collected specimens, and on which host instar is preferred for parasitization, is needed.

The sex ratio of *M. insularis* was determined from parasitoids that emerged from 200 coffee leaves containing larvae and pupae of the coffee leafminer collected from Adjuntas, Puerto Rico (18°10' 54.11" N 66°47' 04.19" W) twice during January 2007. Mined leaves were collected at random and transported to the Biological Control Laboratory (BCL), University of Puerto Rico, Mayagüez Campus, in plastics bags (30 cm × 20 cm). At the BCL the mined leaves were maintained inside a plastic bug dorm® (BD) (insect rearing tent 60 × 60 ×60 cm^3) from two to three weeks, allowing the development of the coffee leafminer. Pupae were removed with a piece of leaf and placed in Petri® dishes (15 × 5 mm) containing wet cotton (in distilled water) and filter paper to maintain the turgency of the leaf piece. The laboratory conditions were 27 ± 1° C, 70% ± 5 RH and 12:12 h of photoperiod. Petri® dishes were observed daily for verifying the emergence of *L. coffeella* or

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M. insularis. Identification was made under a stereoscope. Difference in the sex of *L. coffeella* was determined from the shape of the two last abdominal segments (Nantes and Parra, 1977) whereas *M. insularis* females were differentiated from males by the presence of the ovipositor.

To study the parasitoid host instar preference, we set up an experiment which consisted of four treatments (host instars I, II, III, IV) with four replications. Each replication consisted of six coffee plants inside a BD and previously exposed to 200 moths of *L. coffeella* for oviposition during 48 hours. Each treatment (instar) was distinguished by the number of days afterward oviposition had occurred on coffee leaves. Each BD contained only one instar available (instars I, II, III, IV corresponded to 3, 7, 11 and 13 days post-oviposition, respectively, at laboratory conditions of 27 ± 1° C) (Navarro, 2007). When each instar was estimated to be developed, they were exposed to 150 *M. insularis* adults that were introduced in each BD for 48 hours. After this period adult parasitoids were removed and the larvae were allowed to continue their development inside the leafminer larvae. Adults of *L. coffeella* and *M. insularis* used in this trial were reared from field-collected coffee leaf samples.

Pupae of *L. coffeella* were cut from the leaves and put individually in the petri dishes maintained as before. Parasitoids and leafminers that emerged were counted; if more than one parasitoid emerged from a pupa, it was scored as superparasitism. Dissection of the leafminer larvae was not contemplated as a technique to determine superparasitism because the procedure affects the final number of hosts (pupae) and parasitoids in the sample. For each instar, the percentage of parasitism and the selection coefficient (a measure of instar preference) was calculated according to Cook (1978) and Li et al. (2006).

Treatments were arranged in a randomized complete block design (RCBD), analyzed with ANOVA and means separated by Tukey's multiple range test (INFOSTAT, 2004). The laboratory conditions were 27 ± 1° C, 70% ± 5 RH and 12:12 h of photoperiod.

Table 1 shows the relation between *L. coffeella* adult and parasitoids that emerged from the field samples and their sex ratio. The results show that *L. coffeella* was the dominant emerged species; the sex ratio observed was 53% females and 47% males. For *M. insularis,* 48% of the specimens were female, and 52% male. Sex determination in the order Hymenoptera is based on haplodiploid arrhenotoky, in which males develop from unfertilized eggs and are haploid, whereas females develop from fertilized eggs and are diploid. The mechanism known as single-locus complementary sex determination (sl-CSD) has been observed in some species of Braconidae (Wu et al., 2005). This was not observed in *M. insularis* from our field-collected specimens, where we obtained a close proportion of 1:1 male female. The sex ratio (proportion of females) determined allows us to feel confident that parasitoids recovered from field-collected samples had enough females to conduct the instar preference experiment.

Table 2 shows the percentage of parasitization of *M. insularis* on *L. coffeella*. First and second instars had the highest percentage of parasitism (60% and 63%, respectively), but no parasitism occurred in the third and fourth instars. A significantly higher selection coefficient of *M. insularis* was determined for the second (0.51750) and the first

TABLE 1.—*Percentage of* Leucoptera coffeella *versus* Mirax insularis *and sex ratio from field-collected samples in Puerto Rico.*

Species	Percentage of the total sample	Female (%)	Male (%)
L. coffeella	63	53	47
M. insularis	25	48	52
Not emerged	12	—	—

TABLE 2.—*Percentage of parasitism and selection coefficient of* Leucoptera coffeella *host parasitized by* Mirax insularis[1].

Instar	Percentage parasitism (%)	Selection coefficient
1st	60.00 ± 1.18 a	0.47575 a
2nd	63.00 ± 1.13 a	0.51750 a
3rd	0.07 ± 0.14 b	0.00000 b
4th	0.00 b	0.00000 b

[1]Means followed by same letter in columns (Tukey's Test) do not differ statistically ($P \leq 0.05$).

(0.47575) instars compared to the last two instars. However, no significant differences were detected between the first and second instars. No superparasitism was detected.

Mirax insularis develops in the hemolyph of its host (León, 1997) where it feeds and grows until the pupa stage. According to Li et al. (2006), the nutrition quality may be different in the different host instars. Pennacio et al. (1992) stated that there are differences in the host's quality with increasing age, which affects the developmental performance for the larval parasitoid. Thus, when parasitization has occurred, the parasitoid requires that the host larva remain alive throughout the parasitoid's development (Kuriachan et al., 2006).

Microplitis mediator (Hymenoptera: Braconidae) is another braconid that prefers to parasitize its host's second instars. Li et al. (2006) reported that no *M. mediator* was found in its host's fifth instar. This finding supports the concept that the host's immune system of the fifth instar is strong enough to prevent the development of the parasitoid. This concept may be applied to the results obtained in this study for third and fourth instars of *L. coffeella*. These instars could be strong enough to prevent the development of the parasitoid egg via encapsulation and could overcome the attack of polydnaviruses (PDVs) that are released by female wasps as maternal secretions from their reproductive organs during oviposition (Wharton and Sittertz-Bhatkar, 2002). It is known that these PDVs act to shut down or suppress the host's defenses, allowing the wasp eggs to avoid encapsulation by the host (Stoltz, 1993; Whitfield and Asgari, 2003).

On the basis of the results obtained in this study, the first and second instars are recommended to rear *M. insularis* to optimize the percentage of parasitism and parasitoid development. For mass rearing of the parasitoid, parasitization of *L. coffeella* by *M. insularis* needs to occur between days 3 and 7 post-oviposition. Further studies are needed to determine when it is more appropriate to field-release *M. insularis*. The field liberations should be done when the first and second instars of *L. coffeella* are present in the field.

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