The larval period ranges from 9 to 20 days depending on temper-

After hatching, the larvae immediately start to feed on seed tissues.

laid at the rate of two or three eggs per day for several weeks, pro-

fruit and lays her eggs inside rearing chambers. Normally they are

unpublished). The CBB feeds only on coffee seeds within the berry,

recently been detected in Puerto Rico in August of 2007 (Vega,

tral Africa but has spread to most coffee producing countries in the

world (Hawaii being an exception; Vega et al., 2006), as it has re-

sect have been estimated at $500 million US dollars annually (Vega

As an alternative to cultural and chemical control, a number of
classical biological control programs have been carried out against
the CBB in several countries in Latin America and the Caribbean in
the last 20 years. The African parasitoids Prorops nasuta Waterston,
Cephalonomia stephanoderis Betrem (both Hymenoptera: Bethyl-

1. Introduction

The coffee berry borer (CBB) Hypothenemus hampei (Ferrari) (Coleoptera: Curculionidae: Scolytinae) was

cocenia: Eulophidae) was imported to Mexico. Since then, several studies have

been carried out as part of the post introduction evaluation of this parasitoid. In this paper, information
concerning the parasitism and life-cycle of P. coffea in coffee farms is presented with the objective of pro-
viding information that elucidates its role as a biological control agent. P. coffea showed highly significant
preferences for allocation of two eggs per host, usually one female and one male. Both offspring are able
to develop and reach the adult stage successfully. Lifespan of adults is 2–3 days only. The degree of par-
asitism by P. coffea was more than 95% at the three altitudes tested, when releases consisted of a ratio
of 10 CBB:1 parasitoid. The median survivorship of CBB parasitized by this wasp was 13, 15 and 19 days at
the low, medium and high altitude coffee zones, respectively. The parasitism by P. coffea was higher when
parasitoid releases were carried out simultaneously with the CBB, and decreased with the time between
host and parasitoid releases. We showed that using P. coffea at a density of 1 parasitoid per 10 hosts
resulted in a 3- to 5.6-fold decrease in CBB damage to the coffee seeds when compared to the control.
The importance and value of these results are discussed in terms of the use of P. coffea as a biological con-
trol agent of the CBB in Latin America.

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Eulophidae) have been introduced in almost all coffee producing countries of these two regions (Castillo, 2005; Jaramillo et al., 2005). As a consequence, the biology and ecology of P. nasuta and C. stephanoderis have been extensively studied in the context of their potential as biological control agents (Barrera, 1994; Infante, 1998; Baker, 1999; Damon, 1999; Lauziere et al., 2000; Pérez-Lachaud and Hardy, 2001; Pérez-Lachaud et al., 2002; Batchelor et al., 2006). Although both parasitoids have become established in most countries, their impact on CBB populations has been rather limited and other effective control methods are needed (Damon, 1999; Baker, 1999; Infante et al., 2001). There is another indigenous species, C. hyalinipennis, reported as a parasitoid of the CBB in Chiapas (Pérez-Lachaud, 1998). Further investigations involving this species showed that its use as a natural enemy against the CBB is not feasible, as it has many other coleopteran hosts in the field. In addition, C. hyalinipennis has also been reported as a facultative parasitoid of P. nasuta and C. stephanoderis under laboratory conditions (Pérez-Lachaud et al., 2004). Although the coexistence of these three species in coffee agroecosystems is possible, hyperparasitism by C. hyalinipennis could interfere with the control of the CBB (Pérez-Lachaud et al., 2004; Batchelor et al., 2006). Thus, P. coffea seems to be the most promising species against the CBB.

Phymastichus coffeea is endemic to Africa and appears to be present in most coffee producing countries in that continent. The wasp was first noticed as a CBB parasitoid in Togo in 1987 (Borbón-Martínez, 1989) and later described as a new species (LaSalle, 1990). Parasitoid females are approximately 1 mm long, with males half that size (LaSalle, 1990). In the laboratory the life-cycle of P. coffea takes about 35 days at 26 °C, while the longevity of adults is about 3 days (Feldhege, 1992). This species has been described as a primary, gregarious (usually laying two eggs per host), idiobiont endoparasitoid of adult hosts with a high capacity for host-discrimination (Feldhege, 1992; Infante et al., 1994; Lopez-Vaamonde and Moore, 1998; Castillo et al., 2004a). Recent studies have demonstrated that this wasp uses olfactory cues resulting from the plant-host complex during the host location behavior (Rojas et al., 2006). Since P. coffea is the only known parasitoid of adult CBBs, it potentially has special value when combined with P. nasuta and/or C. stephanoderis, wasps that parasitize immature stages of the CBB (Lopez-Vaamonde and Moore, 1998; Baker, 1999; Gutierrez et al., 1998). The present study provides comprehensive field data on the biology of P. coffea in Mexican coffee plantations. This information will increase the knowledge on the basic biology of this parasitoid, and should help in the development of more effective biological control programs against the CBB.

2. Materials and methods

2.1. Study sites

Experiments related to the life-cycle of P. coffea were conducted at different altitudes in three coffee plantations of Chiapas, Mexico: low zone, Rosario Izapa (N14°57'54"; W92°09'08"; 420 m above sea level (masl); medium zone, La Alianza (N15°02'27"; W92°10'22"; 700 masl), and high zone, Monte Perla (N15°02'48"; W92°05'21"; 920 masl) (Fig. 1). Experiments related to P. coffea parasitism and impact on CBB populations were carried out in Rosario Izapa only.

2.2. The life-cycle of P. coffea

Two coffee plants (Coffeea arabica L.) were selected in each of the three locations. From each plant, two branches were chosen. Two hundred fruits that were not infested by the CBB were selected and the remaining fruits were removed from the branch. Immediately afterwards, an entomological sleeve (30 cm dia, 70 cm long) was placed over the fruits. Fruits were artificially infested with CBB adults reared on meridic diets in the laboratory (Villacorta and Barrera, 1993). In order to obtain a high level of infestation by the CBB, more than 2000 females were released inside each sleeve (i.e., 10 CBB adults per fruit). Three hours after infestation, adult females of P. coffea recently emerged from CBBs reared in the laboratory were placed inside the sleeves at a ratio of 1 parasitoid per 10 hosts. During transportation from the laboratory to the field (usually between 1 and 3 h), parasitoids were fed with honey. The lower parts of the branches were covered with Vaseline® to avoid predation by ants. Sleeves were removed from the plant 15 days after the experiment started to avoid problems with the entomopathogenic fungus Beauveria bassiana (Balsamo) Vuillemin (Ascomycota: Hypocreales), as this fungus can kill the CBB and its parasitoids (De la Rosa et al., 1997; Castillo, 2005).

Following plant infestation, a sample of 25 CBB adults was taken from each location on a daily basis. The CBBs were taken to the laboratory and dissected with entomological pins to observe the development of the immature stages of the parasitoid. Dissections were initiated one day after the parasitoids were released, and finished when the adults of the new generation emerged. Since the length of the life-cycle of P. coffea was different at each site, the total number of samples was also different.

2.3. Parasitism by P. coffea and its impact on CBB populations

Six coffee plants were randomly selected and a branch from each plant with a large number of fruits was chosen; 200 non-infested fruits were selected and the remaining fruits were removed as described above. An entomological sleeve was placed on the branches, and each branch was infested with 2000 CBB adults. Branches were covered with Vaseline® on the lower part of the stem.

On each branch, one of the following six treatments, consisting in the release of 200 female parasitoids inside sleeves at different times, were performed: (i) simultaneously with the CBB, (ii) 2 h after the CBB, (iii) 6 h after the CBB, (iv) 24 h after the CBB, (v) 168 h (1 week) after the CBB, and (vi) control (no parasitoids). The climatic conditions prevailing during the experiment were a mean temperature of 25.6 °C and 60–70% of RH.

Seed damage, survival and reproduction of CBB was estimated daily by dissecting five fruits randomly collected from each treatment. In addition, a sample of 10 CBBs was taken on a daily basis to observe the parasitism of P. coffea. A total of 310 CBBs per treatment were dissected under a stereomicroscope using a pair of entomological pins. The biological material derived from dissections (parasitoids and hosts) was placed in 3% glutaraldehyde and prepared for morphological examination using scanning electron microscopy (SEM).

2.4. Statistical analysis

In all statistical analysis, values of P ≤ 0.05 were considered significant. The observed distribution of the number of eggs found in the CBBs was compared through a Chi-square test for homogenous proportions. A cohort survival analysis using the Cox model was performed to detect differences in CBB survivorship (Cox and Oakes, 1984). To avoid problems of inflated significance rates because of the number of tests, Bonferroni’s correction (Scheiner and Gurevitch, 1993) was applied giving a critical value of α = 0.016. As the data of seed damage by CBB and P. coffea parasitism did not conform to the requirements of a normal distribution, a square root transformation \( \sqrt{x+1} \) was used to meet the assumptions of normality and homoscedasticity (Zar, 1999). After transformation, a univariate analysis of variance (ANOVA) was
conducted and the treatment means were compared using Tukey’s test. The data regarding to the CBB reproduction were subject to a non-parametric analysis using Friedman’s test.

3. Results

3.1. Parasitization by *P. coffea*

More than 95% of CBB adults were parasitized and apparently the parasitic activity of *P. coffea* was not affected by the altitude, as parasitism rates were similar in all three locations, ranging from 95.7% to 97% (Table 1). *P. coffea* was able to parasitize CBB adults immediately after its release inside the sleeves. We noted that female parasitoids landed on the CBB females while they were boring into the coffee fruits and oviposited into the dorsal part of the host abdomen. During oviposition, *P. coffea* holds its wings in a vertical position with respect to its body while grabbing its host with the hind and middle legs. The ovipositor goes through the CBB elytra and it was possible to observe a series of rhythmic movements (pumping) when eggs were deposited inside the abdomen. The parasitoid showed highly significant preferences to lay two eggs per host (usually one male and one female, as discussed below) in the three study locations: Monte Perla ($\chi^2 = 2417, df = 4, P < 0.01$), La Alianza ($\chi^2 = 1761, df = 4, P < 0.01$) and Rosario Izapa ($\chi^2 = 1200, df = 4, P < 0.01$) (Fig. 2). Out of more than 3000 CBB parasitized by *P. coffea*, two eggs per host were deposited in approximately 75% of cases. Host paralysis was never observed.

3.2. Basic morphology of *P. coffea*

The eggs of *P. coffea* have a soft smooth surface and are translucent and elongated. They are approximately 180 $\mu$m long and the anterior pole is slightly wider than posterior pole ($n = 5$) (Fig. 3). The newly emerged larvae are about 250 $\mu$m long, white, rounded, legless, and show little motion. They have a characteristic ornamentation that consists of microscopic protuberances along the body. In small larvae it is not possible to distinguish any segmentation. Fully developed larvae are about 1180 $\mu$m ($n = 5$), white, rounded, legless, slightly curved and have 13 segments. The head is small and the body has neither hairs nor ornamentations. At the beginning of the stage the pupa is whitish, but as grows it becomes dark, being completely black at the end. The pupa is ca. 1148 $\mu$m and exhibits many external structures of the adult parasitoid. Sexes can be differentiated based on pupal size, with females being twice as large than males. Adults of *P. coffea* are black with reddish eyes, with females measuring ca. 1100 $\mu$m in length and males ca. 690 $\mu$m. The thorax of adults is curved, and the wings are shiny with reduced venation.

3.3. Life-cycle of *P. coffea*

The development of immature stages of *P. coffea* at different altitudes is presented in Table 2. The average time for development of *P. coffea* was influenced by temperature, being shorter at Rosario Izapa, where average temperature was higher than at the other two locations. Even though there was only 3 °C in average temperature between the locations with higher (Monte Perla) and lower altitude (Rosario Izapa), there were large differences in the days required for development (11.5 days), indicating that *P. coffea* development times are very sensitive to temperature changes.

The life-cycle of *P. coffea* under the field conditions of Finca Monte Perla (average temperature = 23.2 °C) is presented in Fig. 4. Generally each parasitization by this wasp yielded two eggs, and they incubated in about 5.5 days. After hatching, both larvae

### Table 1

<table>
<thead>
<tr>
<th>Location</th>
<th>Altitude (masl)</th>
<th>Mean temp. (°C)</th>
<th>CBB samples</th>
<th>CBB parasitized</th>
<th>Parasitism (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monte Perla</td>
<td>920</td>
<td>23.2</td>
<td>1200</td>
<td>1149</td>
<td>95.7</td>
</tr>
<tr>
<td>La Alianza</td>
<td>700</td>
<td>24.6</td>
<td>1050</td>
<td>1019</td>
<td>97.0</td>
</tr>
<tr>
<td>Rosario Izapa</td>
<td>420</td>
<td>26.5</td>
<td>900</td>
<td>866</td>
<td>96.2</td>
</tr>
</tbody>
</table>
fed for about 16.7 days on abdominal tissues of the CBB. Differences in the size of the two larvae indicate that both sexes are present in the same host. Female larva always feed in the abdominal region, while the smaller male larva emigrates to the prothorax, where it continues feeding on the internal tissues. It has been proposed that two larvae inside the same host leads to intraspecific competition for food and space (Castillo et al., 2004a). This competition should be more intensive at the end of the larval stage, because the sizes of both larvae occupy most of the internal cavity of the host. This situation ends with the emigration of the weakest larva to the prothorax. The duration of the pupal stage was approximately 22.4 days for both males and females and was the longest life-cycle phase of *P. coffea*. Male pupation occurs in the prothorax, while the female pupates in the abdomen. The male pupa is usually oriented with its head toward the host head, while the female pupa has the head oriented toward the posterior end of the host abdomen. The parasitoids must be able to make an exit hole in the exoskeleton of the CBB in order to successfully emerge. This task is accomplished by the female, who makes a circular hole with its mouth usually on the tip of the CBB abdomen (Fig. 5). Once the female has emerged, the male comes out immediately afterwards using the same hole. Presumably, there is sibling mating just before emergence from the host, but this was not experimentally verified. The life-cycle from egg to adult in *P. coffea* was completed in 47.4 days and the longevity of adults was limited to a maximum of 3 days only.

### 3.4. Survivorship of CBB parasitized by *P. coffea*

The population of CBB parasitized by *P. coffea* started to die one week after the attack, and median survivorship was 13, 15 and 19 days for Rosario Izapa, La Alianza and Monte Perla, respectively. In Rosario Izapa, the CBB population attacked by *P. coffea* did not live longer than 15 days after parasitization, while in La Alianza and Monte Perla, CBB survivorship was somewhat longer; i.e. 21 and 24 days, respectively. There were no significant differences between the survival curves (Fig. 6) of Monte Perla and La Alianza ($\chi^2 = 2.23, df = 1, P = 0.134$). Survival of CBB was significantly shorter at Rosario Izapa than at Monte Perla ($\chi^2 = 21.5, df = 1, P < 0.001$) and La Alianza ($\chi^2 = 13.85, df = 1, P = 0.0002$). As the

---

**Table 2**

Days required for the development of juvenile stages of *Phymastichus coffea* in three coffee plantations located at different altitudes.

<table>
<thead>
<tr>
<th>Location</th>
<th>Altitude</th>
<th>Mean temperature (°C)</th>
<th>Duration of the life-cycle (mean ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Egg</td>
</tr>
<tr>
<td>Monte Perla</td>
<td>920</td>
<td>23.2</td>
<td>5.5 ± 0.57</td>
</tr>
<tr>
<td>La Alianza</td>
<td>700</td>
<td>24.6</td>
<td>4.9 ± 0.91</td>
</tr>
<tr>
<td>R. Izapa</td>
<td>420</td>
<td>26.5</td>
<td>4.1 ± 1.17</td>
</tr>
</tbody>
</table>

---

**Fig. 2.** Distribution of the number of eggs allocated by *Phymastichus coffea* in CBB adults after the release of the parasitoid inside sleeves in three coffee plantations located at different altitudes. A double asterisk indicates a significant difference between expected and observed values within locations.

**Fig. 3.** SEM photographs of biological stages of *Phymastichus coffea*: (a) egg, (b) fully developed larva, (c) pupa and (d) adult.
development of P. coffea is faster in Rosario Izapa, the CBB population died faster than CBBs established in the other locations.

### 3.5. Relationship between the parasitism of P. coffea as a function of its release at different times

The parasitism of P. coffea as a function of its release time is presented in Table 3. The parasitism levels ranged from 67.7% to 86.7%. The highest level of parasitism was obtained when P. coffea was released simultaneously with the CBB ($F = 26.8$, $df = 4$, 120, $P < 0.01$), and decreased as long as the time of parasitoid releases was longer. The lowest percentage of parasitism was obtained when the parasitoid was released 1–7 days after the CBB infestation. These results are in agreement with the expected values, since one day after releases the CBB is expected to be inside the coffee fruit, where there is a lower risk of being parasitized by P. coffea.

### 3.6. Seed damage and CBB reproduction

The damage caused by the CBB to coffee seeds was significantly higher in the control than in the treatments that received releases of parasitoids ($F = 19.7$, $df = 5$, 95, $P < 0.01$). Treatments where P. coffea was released suffered up to 5.6 times less damage in the seed due to CBB than the control (Fig. 7). Reproduction of the CBB was significantly lower in treatments where P. coffea was present than in the control ($\chi^2 = 45.2$, $df = 3$, $P < 0.01$; Fig. 8). Since there was no reproduction of CBB at 0 and 2 h treatments, these were excluded from the analysis. Presumably, the CBB that achieved reproduction were individuals that found refuge inside the coffee fruit, thus escaping from parasitoid attack.

---

**Table 3**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CBB collected</th>
<th>CBB parasitized</th>
<th>Parasitism (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>310</td>
<td>269</td>
<td>86.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 h</td>
<td>310</td>
<td>250</td>
<td>80.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 h</td>
<td>310</td>
<td>226</td>
<td>73.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>24 h</td>
<td>310</td>
<td>210</td>
<td>67.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>168 h</td>
<td>310</td>
<td>212</td>
<td>68.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>310</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ten individuals were collected and dissected daily during 31 days.
<sup>b</sup> Different letters represent significant statistical differences between treatments ($P < 0.01$).
One of the main contributions of the paper is to demonstrate that this parasitoid normally allocates two eggs per host. Although this point has already been suggested by other authors (Borbón-Martínez, 1989; Feldhege, 1992; Infante et al., 1994), they did not provide information on the frequency of the clutch size in this species. We have found that the two individual parasitoids that develop inside the CBB are a female and a male. Similarly, the precise moment in which the male larva of the parasitoid emigrates from the abdomen to the prothorax was established. We have also shown that the female emerges first from the host, followed by emergence of the male, using the same hole that has been carved by the female. However, one aspect about the biology of this parasitoid that was not possible to verify was the number of larval instars. Additional specific experiments to clarify this part of the biology of *P. coffea* are needed.

As we hypothesized, the developmental time of *P. coffea* in the three experimental locations was inversely related to temperature. The successful development of this parasitoid in all locations demonstrates no detrimental effects on its biology. In fact, the range of climatic tolerance for this parasitoid is apparently wider, as it has been released and recovered from many coffee farms of Mexico located between 395 and 1112 masl (Galindo et al., 2002). Our results are in agreement of those of Vergara et al., 2001, who reported that the life-cycle of *P. coffea* in the field was 46 days at a mean temperature 22 °C. If we consider that the life-cycle of this wasp is about 6 weeks for the medium altitude coffee zone (Finca La Alianza), we can predict from 7 to 8 generations for this species throughout a year, if CBB are available, under the climatic conditions of Chiapas.

Previous studies have suggested that the attack by *P. coffea* occurs when the CBB initiates fruit perforation (Borbón-Martínez, 1989; Feldhege, 1992; Lopez-Vaamonde and Moore, 1998). Surprisingly, our study indicates that releases of *P. coffea* at 7 days post-fruit colonization by the CBB resulted in parasitism. Actually we suspect that this species is able to attack at any time after CBB fruit colonization, but no tests beyond 7 days were carried out. The intensity of parasitism decreased by 21% if releases of *P. coffea* are made between 1 and 7 days after the colonization of fruits by the CBB, which demonstrates that coffee fruits are used as a spatial refuge to escape from parasitism. In this sense, our results are similar to those reported by Echeverry (1999) and Jaramillo et al. (2006) who mentioned that this wasp is able to attack CBBs that have already entered and colonized coffee fruits. It is not completely clear how parasitization of CBB inside fruits occurs. It is not known whether *P. coffea* is able to enter the coffee fruit searching for its host, or if the wasp stays outside the fruit waiting for the appearance of the CBB when is cleaning the gallery. In any case, this searching behavior could have important practical implications because *P. coffea* could be released in any vegetative period of the coffee plant, as long as there are available infested fruits on the plant.

Our results demonstrate that using *P. coffea* at a density of 1 parasitoid per 10 hosts results in a 3–to 5.6-fold decrease in CBB damage to the coffee seeds when compared to the control. This is due to the fact that individuals parasitized by *P. coffea* drastically change their behavior, stop reproducing and die before they damage the coffee seed (Castillo, 2005). We believe that the few CBBs that damage the coffee seeds and produced progeny in treatments with parasitoids (see Fig. 7 and 8), were individuals that escaped *P. coffea*, based on the fact that none of the treatments yielded 100% parasitism. Even though the classical biological control of the CBB using the bethylids *C. stephanoderis* and *P. nasuta* in Latin America has not been successful (Infante, 1998; Damon, 2000; Jaramillo et al., 2006) and conservation biological control using *C. hyalinipennis* also does not seem possible (Batchelor et al., 2006), the recent introduction of *P. coffea* to this region holds new expectations for the biological control of the CBB (Castillo, 2005; Jaramillo et al., 2005). One important point is that the persistence and efficacy of these species of parasitoids in coffee agroecosystems could be improved by the use of repetitive mass releases throughout the coffee season. In this case, laboratory parasitoid production could be based on techniques already established where the CBB is reared in artificial diets and later used as a host to produce parasitoids (Villacorta and Barrera, 1993; Portilla, 1999). An interesting method for mass rearing programs has been proposed by Pérez-Lachaud and Hardy (2001), who recommend the use of factitious hosts, such as, *Caulophilus oryzae* (Cylindrical) and two species of *Sitophilus* sp. to produce *C. stephanoderis* and *P. nasuta*. Factitious hosts have also been reported for *P. coffea* (Castillo et al., 2004b), thus opening the possibility of rearing this species on other hosts.

To conclude, our study is an attempt to provide new information on the biology and ecology of *P. coffea*. A better understanding of these aspects of natural enemies of insect pests has direct applications in biological control programs. So far, the results in the use of an entomopathogenic fungus, African (*C. stephanoderis* and *P. nasuta*) and native (*C. hyalinipennis*) parasitoids have been disapp...
pointing and hope rests with \textit{P. coffea} (Damon, 2000). Our findings indicate that \textit{P. coffea} is a promising biological control agent against the CBB, perhaps not as a single method of control, but as an important component of a pest management program. However, it is essential to accurately assess, for every particular coffee producing country of Latin America, the role of each species of African parasitoids in the population dynamics of the CBB. This would be an important step in determining their importance as regulatory factors and to develop strategies to enhance their impact on CBB populations.

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