Field superparasitism by *Phymastichus coffea*, a parasitoid of adult coffee berry borer, *Hypothenemus hampei*

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Abstract

Superparasitism by *Phymastichus coffea* LaSalle (Hymenoptera: Eulophidae), a parasitoid of adults of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), was recorded under field conditions in a coffee plantation in Colombia. Parasitoid adults were released 1, 5, and 9 days after artificial infestations of 90-, 150-, and 210-day-old coffee berries with *H. hampei* females. The position of the beetle inside the berry and the number of *P. coffea* larvae per female host were assessed 10 days after each parasitoid release. Under laboratory conditions, *P. coffea* usually lays two eggs per host, one female and one male. In our studies, we often recorded more than six *P. coffea* larvae in an individual host and mean numbers of larvae per host ranged from two to 4.45. Superparasitism by *P. coffea* under field conditions was influenced by the age of the coffee berries, which is the most important factor determining the speed of penetration by *H. hampei*, and therefore the time the beetles are exposed to a *P. coffea* attack. The number of parasitoid larvae in each *H. hampei* female gradually decreased with the age of the berry, and also linearly decreased with the time of parasitoid release. Age-dependent effects of coffee berries that alter the ratio of available hosts to searching parasitoids by providing refuges to the herbivore, largely determine the extent of superparasitism of *H. hampei* by *P. coffea* under fields conditions in Colombia.

Introduction

*Phymastichus coffea* LaSalle (Hymenoptera: Eulophidae) is a gregarious endoparasitoid of females of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), the most important pest of commercial coffee worldwide (LePelley, 1968). In Colombia, *H. hampei* was initially recorded in 1988, is presently widespread throughout all coffee-growing regions of the country, and is considered to be the country’s number one pest (Baker, 1999). *Phymastichus coffea* was found in Togo, West Africa in 1987 (Borbón, 1989). It parasitizes *H. hampei* females when they start boring into the berries (Lopez et al., 1997; Jaramillo et al., 2005), which prevents further penetration of the beetles into the coffee berries and as a consequence damage to the endosperm. *Phymastichus coffea* females start to search for their hosts immediately after emerging from the *H. hampei* mummy; parasitization of *H. hampei* can occur within the first hours after emergence. According to Infante et al. (1994), *P. coffea* females lack a preoviposition period, whereas Feldhege (1992) reported preoviposition periods between 5 min and 4 h, with 20 min as the most frequent duration. Female *P. coffea* oviposits into the abdomen, thorax, or between the thorax and the abdomen of a *H. hampei* female (Feldhege, 1992). In the laboratory, honey-fed *P. coffea* females normally live for 2–3 days (Infante et al., 1994). The parasitization behaviour of *P. coffea* under field conditions remains unknown. Under laboratory conditions, *P. coffea* females always lay two eggs into their hosts; one female offspring develops in the abdomen of the beetle, whereas...
the male larva migrates to the head and completes its development there (Infante et al., 1994; Lopez & Moore, 1998). After parasitization, the mobility of the *H. hampei* female is impaired; it stops oviposition and the beetle usually dies after 12 days (Feldhege, 1992; Infante et al., 1994). In 1997, *P. coffea* was released for the first time in Colombia and its establishment was reported 1 year later (Baker, 1999). Parasitism in the field is strongly influenced by several factors such as the developmental stage of the *H. hampei*-infested berries, i.e., its dry matter content, and the position of *H. hampei* inside the berry at the time of parasitoid release (Jaramillo et al., 2005). The same authors recorded levels of parasitism in the field of up to 85% in a coffee plantation in Colombia, confirming the potential of *P. coffea* for biological control of *H. hampei* (Baker, 1999).

Most parasitoids are able to recognize hosts previously parasitized by themselves or by a conspecific female (host discrimination) (van Lenteren, 1981). However, superparasitism, i.e., a female parasitoid that oviposits an egg or a clutch of eggs in a host already parasitized by a female of the same species (conspecific superparasitism) or by herself (self-superparasitism), is a common phenomenon in nature (van Alphen & Visser, 1990). Superparasitism may be adaptive in several circumstances (Visser et al., 1990), for instance, when there is a high risk of encapsulation (in the case of solitary endoparasitoids) or when there is a high chance of a later attack by a conspecific female (van Alphen & Visser, 1990). The decision whether to superparasitize seems to be mediated not only by the physiology of the female parasitoid itself, i.e., its life expectancy (Sirot et al., 1997), egg load [with decreasing egg loads, parasitoid females are more reluctant to lay eggs in already parasitized hosts (Sirot et al., 1997; Islam & Copland, 2000)], or the quality of hosts encountered (Waage & Godfray, 1985; Goubault et al., 2004), but also by other factors such as previous experience of competition (Hoffmeister et al., 2000), the numbers of competitors simultaneously entering the patch and the number of unparasitized hosts available there. Consequently, superparasitism becomes more likely with increasing numbers of female parasitoids searching for a limited number of hosts (van Alphen & Visser, 1990). Under natural conditions, intraspecific competition is predicted to influence clutch size (Visser & Rosenheim, 1998). A female (or group of females) encountering few healthy hosts might assess the habitat as poor and thus be more willing to superparasitize (Visser et al., 1990). In the case of *P. coffea*, Castillo et al. (2004) observed under laboratory conditions that females are able to discriminate between parasitized and non-parasitized hosts in choice experiments, whereas under no-choice conditions, females superparasitized *H. hampei* females. As little is known about superparasitism by *P. coffea* under field conditions, the objective of this study was to investigate the behaviour of the parasitoid in a commercial coffee plantation in Colombia. Moreover, the effects of the position of *H. hampei* inside the coffee berries and the release ratio of parasitoids to hosts on the clutch size are studied.

**Materials and methods**

**Study site**

The study was carried out on an experimental coffee plantation of the Centro Nacional de Investigaciones de Café (CENICAFE) near Chinchiná, Colombia (latitude 04°59’N; longitude 75°39’W; 1400 m above sea level; 21.4°C mean annual temperature; 2700 mm precipitation per year; 80% mean r.h.). This coffee plantation had previously not been treated with synthetic insecticides, nor had parasitoids of *H. hampei* been released there. However, cultural control practices such as a rigorous removal of *H. hampei*-infested coffee berries were routinely performed to mimic normal coffee growing conditions in Colombia. Climatic data, i.e., temperature, relative humidity, solar radiation, and precipitation, were measured daily during the course of the study.

**Origin of Hypothenemus hampei and Phymastichus coffea females**

The *H. hampei* females used in this study were obtained from the entomology department of CENICAFE where they are mass reared following the protocol developed by Bustillo et al. (1998). For the experiment, *H. hampei* females were collected in the rearing facility, transferred to plastic boxes filled with staple paper, and then brought to the field. *Phymastichus coffea* females originated from a stock culture maintained at CENICAFE. There, the parasitoids are mass produced using plastic boxes filled with *H. hampei*-infested parchment coffee of 45% moisture content. The boxes are then kept under controlled conditions (25°C, 75% r.h., and complete darkness) until the development of *P. coffea* is completed. Once the parasitoids are ready to emerge, the boxes are taken to an emergence chamber equipped with a fluorescent light. Because of the positive phototaxis of *P. coffea*, they tend to concentrate near the lamp and can be easily collected with a vacuum pump. Female parasitoids were then introduced into plastic vials, covered with muslin impregnated with a honey-water solution, and transported to the field.

**Experimental procedure**

A 5-year-old *Coffee arabica* (L.) cv. Colombia plantation with 650 trees (1 × 1 m planting distance) was selected for the experiment. An experimental plot was defined as nine trees arranged in a 3 × 3 square, and a total of 72 experimental plots were established. The central tree was labelled and served as the sampling unit. Because of the precipitation pattern in the coffee-growing area of Colombia, berries of
different physiological stages may be found in the same branch or tree (Arcila et al., 2001). A heavy rain following a prolonged dry period usually triggers the blossoming of the coffee tree (Trojer, 1968). Therefore, on every branch of the selected trees (sampling units), all berries and already open flowers were removed and only new flowers were kept on the branches, assuring a subsequent high degree of uniformity of the berries during the course of the experiment. One branch with 50 healthy flowers per tree was selected and labelled. Subsequently, 50 coffee berries 90, 150, and 210 days after flowering were artificially infested with *H. hampei* females, mimicking the infestation pattern of *H. hampei* in coffee berries of different ages (Salazar et al., 1993). For this, the selected branches were covered with an entomological sleeve, and 250 *H. hampei* females were introduced per branch. Each berry is normally attacked by one female *H. hampei*. The sleeves were removed 24 h later, assuring a 100% infestation of the berries by *H. hampei*. Thereafter, 50 *P. coffea* were released around each infested branch. The host/parasitoid release ratio was 1 : 1, based on the numbers of *H. hampei* in 50 infested berries. Parasitoids were released at three intervals, i.e., 1, 5, and 9 days after the artificial *H. hampei* infestation, to the branches holding coffee berries of the three different age classes. The 4-day interval between the three release times (treatments) prevented parasitoids from different treatments to parasitize or superparasitize *H. hampei* females from previous treatments, as under laboratory conditions honey-fed *P. coffea* females live only for up to 3 days (Infante et al., 1994). Nine treatments based on the combinations of the age of the berries and the time of the *P. coffea* releases after the initial *H. hampei* infestation were evaluated using eight trees per treatment. The number of *P. coffea* larvae per host was assessed 10 days after each release of the parasitoids. For this, all berries of a selected branch were collected and dissected, and the position of *H. hampei* inside the berry was assessed. According to Bustillo et al. (1998), the positions of the *H. hampei* female in the coffee berry are defined as: position A, when *H. hampei* is starting the colonization of a new berry and the penetration of the exocarp begins; position B, when *H. hampei* has started penetrating the berry but has not yet reached the endosperm; position C, when the beetle has started to bore into the endosperm but has yet not commenced oviposition; and position D, when *H. hampei* has produced a gallery in the endosperm, and one or more of its immature stages are found inside the gallery. After recording the position of the *H. hampei* female inside the berry, the beetle was removed from the berry, placed on a glass slide under a stereomicroscope (40 × magnification) and dissected, and the number of *P. coffea* larvae inside *H. hampei* were counted.

**Statistical analysis**

For each combination of the coffee berry age classes and release times of *P. coffea* (treatments), the numbers of *P. coffea* larvae inside the *H. hampei* female for a given position of the beetle inside the coffee berries (positions A–D) were recorded. The number of parasitoid larvae inside *H. hampei* females across the age of the berries (time of artificial infestation with *H. hampei* females) and times of *P. coffea* release were compared with a general linear model using the SAS procedure GENMOD, with Poisson distribution and log link function. Pairwise comparisons of the means were obtained using the LSMEANS procedure within SAS (SAS, 1996).

**Results**

In Figure 1, data on the distribution of *H. hampei* females inside coffee berries are presented across the different age
classes of the berries, as well as the time delay between artificial infestation of coffee berries with *H. hampei* females and the subsequent releases of *P. coffea*. Results clearly indicate that the proportion of beetles at positions A and B are considerably greater in younger compared to older berries and that in general more beetles were found deeper inside the coffee berries with increasing time between artificial infestation of the berries with *H. hampei* females and the subsequent releases of the parasitoids (Figure 1).

All three variables, i.e., age of the coffee berries/age of artificial infestation with *H. hampei* 

\( \chi^2 = 46.90, \text{d.f.} = 2, P < 0.0001 \), time of parasitoid release \( \chi^2 = 223.22, \text{d.f.} = 2, P < 0.0001 \), and position of the beetles inside the berries \( \chi^2 = 13.01, \text{d.f.} = 3, P = 0.005 \) significantly affected the number of eggs *P. coffea* females oviposited in *H. hampei* females. The number of parasitoid larvae in each *H. hampei* female gradually decreased with the age of the berry in which the beetle host was feeding. Similarly, the number of parasitoid eggs deposited per host linearly decreased with the time of parasitoid release, from 1 to 9 days after coffee berry infestations with *H. hampei* (Figure 1).

The number of parasitoid larvae per *H. hampei* at positions A and B was significantly higher than at positions C and D \( \chi^2 = 438.97, \text{d.f.} = 3, P < 0.001 \). Significant differences in the numbers of *P. coffea* larvae per host were recorded among coffee berry age classes as well as among the three times of *P. coffea* release (Table 1). The numbers of *P. coffea* larvae inside the hosts differed significantly between positions A and C \( \chi^2 = 6.71, \text{d.f.} = 1, P = 0.0096 \) and positions B and C \( \chi^2 = 9.76, \text{d.f.} = 1, P = 0.0018 \). However, no significant differences were found between positions A and B \( \chi^2 = 1.59, \text{d.f.} = 1, P = 0.2074 \), A and D \( \chi^2 = 1.41, \text{d.f.} = 1, P = 0.2347 \), B and D \( \chi^2 = 0.20, \text{d.f.} = 1, P = 0.6544 \), and C and D \( \chi^2 = 0.83, \text{d.f.} = 1, P = 0.3633 \). High numbers of parasitoid larvae per beetle host were recorded following releases of *P. coffea* females to *H. hampei* attacking 90-day-old coffee berries (Figure 2). Moreover, when *P. coffea* were released 1 day after the *H. hampei* infestation in this berry age class, high numbers of parasitoid larvae were found in hosts at position A (4.4), B (4.5), and C (3.2) (Figure 2). When parasitoids were released 5 and 9 days after the *H. hampei* infestation, however, no hosts were found at positions A and D, and the number of parasitoid larvae per host at positions B and C were 2.3 and 2.2, respectively.

Likewise, releases of *P. coffea* 1 and 5 days after the artificial infestation of 150-day-old coffee berries by *H. hampei* resulted in high numbers of parasitoid larvae per host female at positions B and C (Figure 2). Mean numbers of larvae per host were 3.0 and 3.1, and 2.2 and 2.3 for releases carried out 1 and 5 days after the artificial infestation with *H. hampei* at positions B and C, respectively (Figure 2). In 210-day-old berries, two *P. coffea* larvae were always found per host, independent of the positions of *H. hampei* inside the berries and the release times of the parasitoids (Figure 2).

![Figure 2](image_url)  
**Figure 2** Number of *Phymastichus coffea* larvae per host (mean + SD) found in *Hypothenemus hampei* adults attacking 90-, 150-, and 210-day-old coffee berries following parasitoid releases 1, 5, and 9 days after artificial infestation of the coffee berries; positions A–D refer to the depth of penetration of *H. hampei* into the coffee berries (for details see text).

<table>
<thead>
<tr>
<th>Effect</th>
<th>d.f.</th>
<th>( \chi^2 )</th>
<th>( P &gt; \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of coffee berries/infestation with <em>H. hampei</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 days/150 day</td>
<td>1</td>
<td>15.82</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>90 days/210 days</td>
<td>1</td>
<td>44.69</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>150 days/210 days</td>
<td>1</td>
<td>19.88</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time of <em>P. coffea</em> release</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day/5 days</td>
<td>1</td>
<td>127.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1 day/9 days</td>
<td>1</td>
<td>180.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>5 days/9 days</td>
<td>1</td>
<td>4.57</td>
<td>0.0325</td>
</tr>
</tbody>
</table>

![Table 1](table_url)  
**Table 1** Results of LSMEANS pairwise comparison for number of *Phymastichus coffea* larvae found per female *Hypothenemus hampei* attacking 90-, 150-, and 210-day-old coffee berries following parasitoid releases 1, 5, and 9 days after artificial infestation of the coffee berries.
Considerable levels of superparasitism of *H. hampei* by *P. coffea* were recorded under field conditions in Colombia, depending on the age of the coffee berries, the positions of the beetles inside the berries and the time of parasitoid releases. According to Castillo et al. (2004), in choice experiments *P. coffea* discriminates between parasitized and unparasitized hosts; however, under no-choice conditions the authors recorded superparasitism when the females were exposed to *H. hampei*, thus corroborating our field observations. In the field, various factors such as patch quality (van Alphen & Visser, 1990), food availability (Harvey et al., 2001), and the physiology of the female parasitoid, including its egg load (Babendreier & Hoffmeister, 2002) and life expectancy (Sirot et al., 1997), should influence the extent of superparasitism. In our study, factors such as the dry matter content of the coffee berries and the host/parasitoid release ratio might explain the levels of superparasitism recorded in the field.

High numbers of *P. coffea* larvae in *H. hampei* females that attacked 90- and 150-day-old coffee berries were often recorded, especially when parasitoids were released 1 and 5 days after the artificial infestations of the coffee berries with the beetles. Nothing is known about egg cannibalism in *P. coffea* larvae. Moreover, as we dissected the *H. hampei* females 10 days after the releases of the parasitoid, we cannot rule out a potential contribution of egg predation to the number of parasitoid larvae recorded inside the beetles. However, we believe that physical effect of the berries, as a result of their dry matter content, is the main factor explaining the extent of superparasitism in *H. hampei* females by *P. coffea*, as it influences the pattern of attack and the speed of penetration of *H. hampei* in the coffee berries, and thus the availability of hosts for *P. coffea* (Salazar et al., 1993; Ruiz, 1995; Jaramillo et al., 2005).

In this study, superparasitism, either self or conspecific, was recorded when *P. coffea* was released at a time when *H. hampei* had just commenced penetrating the coffee berries (positions A and B), and was thus exposed to a parasitoid attack, confirming previous observations that *P. coffea* can only parasitize *H. hampei* females as long as the beetles have not penetrated deep into the berries (Lopez & Moore, 1998; Jaramillo et al., 2005). In this case, not only the number of parasitoids released but also the availability of hosts considerably influenced the extent of superparasitism of *H. hampei* by *P. coffea*. In general, superparasitism increases when many female parasitoids explore a patch containing only a limited number of healthy hosts (van Alphen & Visser, 1990), and rejection of parasitized hosts is more frequent when unparasitized hosts occur in high numbers in a patch (van Lenteren, 1981). However, in *H. hampei* and *P. coffea*, it is not so much the density of hosts that influences superparasitism but their physical availability, i.e., female beetles at positions A and B. The extent of the latter depends on the age of the coffee berries. Increasing age of the berries leads to a decrease in the time between initial penetration of the berries and oviposition by *H. hampei* (Ruiz, 1995). Hence, in more mature berries, *H. hampei* females rapidly penetrate into the endosperm and are then no longer exposed to an attack by *P. coffea* as the parasitoid can not penetrate into the coffee berry (Jaramillo et al., 2005). This could explain the superparasitism in *H. hampei* attacking 90- and 150-day-old berries compared to the virtual absence of superparasitism in mature berries at 210 days after flowering. Parasitism recorded in beetles attacking 210-day-old berries following releases 5 and 9 days after infestation by *H. hampei* can possibly be explained by the guarding behaviour of the female beetles. For instance, during dissections of berries in the laboratory, 64% of the females that had already produced offspring inside the berries, i.e., at position D, were found at position B (Jaramillo, unpubl.). Probably, these females were blocking the entrance of the galleries to bethylid parasitoids such as *Prorops nasuta* Waterston and *Cephalonomia stephanoderis* Betrem, which would eventually attack their brood (Lauzière et al., 2000; Infante et al., 2005), but at the same time by doing so, exposing themselves to parasitism by *P. coffea*.

Effects of host plants on natural enemies have been extensively studied. Host plant traits, such as morphology, plant nutrition, leaf mineral content (Jiang & Schulthess, 2005; Sétamou et al., 2005), and plant architecture and phenology (Martin et al., 1990), may have direct or indirect effects on natural enemies, influencing their search for hosts/prey or their successful establishment (Bottrell et al., 1998). Likewise, host plant compounds might influence natural enemies in general, and parasitoids in particular. For instance, Ode et al. (2004) demonstrated how plant chemistry may affect parasitoid traits like body size, sex allocation decisions, and clutch size.

Our results show a physical effect of the host plant on superparasitism by *P. coffea*. Theoretical models predict that when the patch is depleted, i.e., when unparasitized hosts become less frequent, superparasitism becomes an adaptive strategy (van Alphen & Visser, 1990). Under the conditions of our study, the patch should be considered depleted not only when few unparasitized hosts remain in the patch, but also when the hosts are inside the coffee berries and hence out of reach for *P. coffea*. In this case, a more adaptive strategy would be to leave the patch and search for unparasitized hosts elsewhere. Vergara et al. (2001) reported 31% parasitism in *H. hampei* females attacking coffee berries at 60-m distance from the parasitoid release point in a commercial coffee plantation in Colombia. The
results of our study show that age-dependent effects of coffee berries that alter the ratio of available hosts to searching parasitoids by providing refugees to the herbivore, largely determine the extent of superparasitism of \textit{H. hampei} by \textit{P. coffea} under field conditions.

An additional factor that might have contributed to the extent of superparasitism by \textit{P. coffea} is the host/parasitoid release ratio. Presently, little is known on optimal host/parasitoid release ratios for \textit{P. coffea} and \textit{H. hampei} under field conditions, and thus a ratio of 1 : 1 was used in our experiments. Superparasitism is more frequent when high numbers of female parasitoids explore a patch simultaneously (van Alphen & Vet, 1985), because the decision to stay longer in the patch and superparasitize is strongly influenced by the presence of competing conspecifics (van Alphen & Vet, 1985), which eventually affects clutch sizes (Visser & Rosenheim, 1998). The latter authors reported that the clutch sizes of females kept individually in the laboratory before the experiments were lower than the ones kept with conspecifics, and speculated that under field conditions an even stronger response might be expected. The \textit{P. coffea} used in our study were collected from a mass rearing, transported in groups of 50 females to the field, and released simultaneously around the \textit{H. hampei}-infested branch. Thus, our results are in line with expectations of Visser & Rosenheim (1998).

In conclusion, for future mass releases of the parasitoids in coffee plantations, the host/parasitoid release ratio should be optimized according to the physiological state of the coffee berries at the time of releases.

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References


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