Biological control of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae) by *Phymastichus coffea* (Hymenoptera: Eulophidae) in Colombia

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Abstract

The potential of the eulophid parasitoid *Phymastichus coffea* LaSalle to control coffee berry borer *Hypothenemus hampei* (Ferrari) populations under field conditions in Colombia was evaluated. Parasitoid adults were released one, five and nine 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 parasitism levels were assessed ten days after each *P. coffea* release. Parasitism of *H. hampei* by *P. coffea* was significantly affected by the age of the berries at the time of infestation, and by the position of the beetle inside the berries. Highest levels of parasitism were recorded in 150-day-old berries (75–85%) and in 90-day-old berries (75%) when *P. coffea* were released one day after the artificial infestation with *H. hampei*. In 150-day-old berries, highest levels of parasitism were recorded for *H. hampei* found in the outer layer of the endosperm followed by beetles penetrating the exocarp. Increasing the time of *P. coffea* releases after the artificial infestations with *H. hampei* led to decreased levels of parasitism in beetles attacking 90- and 150-day-old coffee berries. Low levels of parasitism were recorded in *H. hampei* females infesting older coffee berries because most of the beetles had already constructed galleries deep in the endosperm of the berries, i.e. out of reach of the parasitoid. The potential of *P. coffea* for biological control of coffee berry borer in Colombia is discussed.

Keywords: coffee berry borer, *Hypothenemus hampei*, parasitoids, *Phymastichus coffea*, coffee, biological control, Colombia

Introduction

The coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae) is the most important coffee pest worldwide (Le Pelley, 1968; Damon, 2000). It was accidentally introduced to South America in 1913 from its native region in central Africa (Bergamin, 1943), and has since become the main threat to coffee production in several countries including Brazil, Guatemala, Honduras, Ecuador, El Salvador and Bolivia (Bustillo, 2002). In Colombia, *H. hampei* was first recorded in the southern part of the country in August 1988 (Bustillo *et al*., 1998). To date, it is widespread throughout all coffee-growing regions of Colombia and is

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considered to be the country's number one pest, causing serious economic losses and affecting the economy of more than half a million families in Colombia (Bustillo *et al*., 1998). Under low pest pressure the conversion factor between freshly harvested coffee berries and parchment coffee is 5 : 1; however, a serious infestation can alter this ratio up to >17 : 1 (Baker *et al*., 2002).

Females of *H. hampei* bore galleries into the endosperm of the coffee berries causing two types of damage, premature fall of berries younger than 80 days (Decazy, 1990) and qualitative and quantitative losses in coffee through feeding of the larvae inside the berries (Damon, 2000). Usually a berry is attacked by only one female, the latter often referred to as founder or colonizing female. After the start of oviposition the female wing muscles degenerate, preventing her from colonizing other berries (Ticheler, 1963). The population dynamics of and the infestation pattern by *H. hampei* are closely related to the physiology of the coffee plants. The dry matter content of the endosperm, which increases with age of the fruits, is the most crucial factor determining the attack by *H. hampei* (Salazar *et al*., 1993). Coffee berries with seeds <20% dry matter content are either abandoned after an initial attack, or the female waits in a tunnel bored into the exocarp until the endosperm has accumulated a sufficient amount of dry matter content for the development of her offspring (Alonzo, 1984; Ruiz, 1995).

Initially, Colombian coffee growers tried to combat *H. hampei* infestations predominantly through use of broad-spectrum insecticides. However, growing environmental concerns and increasing problems with insecticide resistance (Gongora *et al*., 2001) stimulated the search for more environmentally friendly control strategies against the coffee berry borer in Colombia. As an exotic outbreak pest in South America, a classical biological control approach was pursued. Initially, two larval–pupal ectoparasitoids of *H. hampei*, the bethylids *Cephalonomia stephanoderis* Betrem and *Prorops nasuta* Waterston, which were found in Ivory Coast (Ticheler, 1963) and Uganda (Hempel, 1934), respectively, were introduced to Colombia in the late 1980s (Baker, 1999). They were first tested under laboratory conditions and subsequently released in the field (Benavides *et al*., 1994; Portilla & Bustillo, 1995). Although both parasitoids successfully established in Colombia, their impact on field populations of *H. hampei* has been rather limited (Quintero *et al*., 1998).

In 1996 a third parasitoid of *H. hampei*, the eulophid *Phymastichus coffea* LaSalle, was introduced to Colombia. *Phymastichus coffea* was found in Togo in 1987 (Borbón, 1989) and described by LaSalle (1990). It is a gregarious endoparasitoid of *H. hampei* females (Borbón, 1989), and usually one male and one female *P. coffea* develop inside each host (Lopez & Moore, 1998). After being parasitized, the mobility of the *H. hampei* female is impaired and parasitized females stop oviposition and usually die after 12 days (Feldhege, 1992; Infante *et al*., 1994). At 23°C the life cycle of *P. coffea* is 43 days and the lifespans of males and females are 1–2 and 3–4 days, respectively (Lopez *et al*., 1997). Since 1996, *P. coffea* has been mass reared at the Centro Nacional de Investigaciones de Café (CENICAFE), in Chinchiná, Colombia, and basic biological studies of the parasitoid have been conducted (Vergara *et al*., 2001). In 1997 *P. coffea* was released for the first time in Colombia and its establishment was reported in 1998 (Baker, 1999). Further releases followed and, according to Aristizabal *et al*. (2004),

P. coffea has established to date on 41 farms in Colombia. The present study reports for the first time the impact of *P. coffea* on field populations of *H. hampei* following releases in a coffee plantation in Colombia.

Materials and methods

Study site and experimental plot

Experiments were carried out between January and October 2001 on the experimental coffee plantation 'Naranjal-Cenicafé' (latitude 04°59′ N; longitude 75°39′ W; altitude 1400 m; 21.4°C mean annual temperature; 2700 mm precipitation/year; 80% mean relative humidity) near Chinchiná, Colombia. A five-year-old *Coffea arabica* (L.) cv. Colombia plantation with 650 trees (1×1 m planting distance) was chosen 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. The coffee crop had not previously been treated with synthetic insecticides nor had parasitoids of *H. hampei* been previously released there. However, 'Re-Re' the cultural control practice against coffee berry borer recommended by CENICAFE (Bustillo *et al*., 1998), which consists mainly of a rigorous removal of infested coffee berries, was routinely performed in the crop, mimicking normal coffee growing conditions in Colombia. Climatic data including temperature, relative humidity, solar radiation and precipitation, were measured daily during the course of the study.

Insects

Females of *H. hampei* used in this study were obtained from the CENICAFE stock colony in Chinchiná, where they are mass reared using re-hydrated premium quality parchment *C. arabica* cv. Colombia with 45% moisture content, under controlled conditions, 24±1°C, 80% relative humidity (RH) and complete darkness (Bustillo *et al*., 1998). On the day the coffee plants were artificially infested, *H. hampei* of mixed age were collected in the rearing unit, transferred to plastic boxes filled with staple paper, and then brought to the field.

Adults of *P. coffea* used in the experiments also originated from the CENICAFE rearing unit. They are mass-produced on *H. hampei*-infested parchment beans, following the protocol developed by Orozco (2002). In the present study, immediately after emergence from the *H. hampei* mummies, the female parasitoids were introduced into plastic vials, and covered with tulle impregnated with a honey-water solution. The vials were then placed in a cool box and transported to the field. Only adults of no more than one hour after emergence were used in the experiment.

Artificial infestations with H. hampei and P. coffea releases

The experiment was initiated in the last week of February 2001, during the main blossoming of the coffee crop for the subsequent main harvest in October (Salazar *et al*., 1994). On every branch of the selected trees (sampling units) all berries and already open flowers were removed and only new flowers kept, assuring a subsequent uniformity of the berries during the experiment. One branch in the middle of each tree that had at least 50 healthy flowers was labelled. Coffee trees were infested at 90, 150 and 210 days after blossoming,

Table 1. ANOVA results for parasitism of coffee berry borer (CBB) *Hypothenemus hampei* adults by *Phymastichus coffea* released one, five and nine days after infestation with *H. hampei* adults on 90-, 150- and 210-day-old coffee berries.

Source of variation	df	F	P
Age of berries	2	28.76	< 0.0001
Position of CBB	3	316.51	< 0.0001
Age × position	6	54.62	< 0.0001
Time of *P. coffea* release	2	0.97	0.3796
Age × release	4	1.39	0.2395
Release × position	6	7.70	< 0.0001
Age × release × position	12	12.98	< 0.0001
Error	252	–	–

mimicking the infestation pattern of coffee berry borer in berries of different ages (Salazar *et al.*, 1993). During these periods the mean dry matter content of coffee berries are 22.4, 268.9 and 514.9 mg in 90-, 150- and 210-day-old berries, respectively (Salazar *et al.*, 1994). The selected branches were then covered with an entomological sleeve, and artificially infested with 250 females of *H. hampei* per branch, following the methodology described by Villalba *et al.* (1995). After 24 h the sleeves were removed, assuring a 100% infestation of the berries. The parasitoids were released around the selected branch in a ratio of 1:1 to the number of *H. hampei* infested berries per branch, i.e. 50 *P. coffea* per tree. For each age of infestation, parasitoids were released at three different intervals, i.e. one, five and nine days after the artificial infestation of the branches with *H. hampei*. Consequently, nine treatments based on the combinations of the age of the berries and the time of the *P. coffea* releases after the initial infestation with *H. hampei* were evaluated using eight trees per treatment. Parasitism was assessed ten days after each release of *P. coffea*. For this, all berries of the selected branches were collected and taken to the laboratory where the berries were dissected and then the position of the beetle inside the berry was recorded. The positions of the *H. hampei* female in the coffee berry were defined as: position A, when the beetle is starting the colonization of a new berry and the penetration of the exocarp begins; position B, when the beetle 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 not yet commenced oviposition; and position D, when the beetle has produced a gallery in the endosperm, and one or more of its immature stages are found inside the gallery (Bustillo *et al.*, 1998). Once the position of the beetle was recorded, it was removed from the berry and dissected to detect the immature stages of *P. coffea* inside the abdomen of *H. hampei*.

Statistical analysis

For each combination of the age of the berries and the release times of *P. coffea*, i.e. treatments, the rate of parasitism for a given position of the beetle inside the coffee berries was calculated as the ratio between the numbers of parasitized and total *H. hampei* found at this particular position. To precisely assess the interaction effects of the position of the beetle inside the berries (a categorical variable) with the other continuous variables (the time of *P. coffea* releases and the age of the berries at the time of infestation, with *H. hampei*), parasitism levels were evaluated using three- or two-way analysis of variance (ANOVA) using the PROC

GLM procedure of SAS (SAS, 1996). In case ANOVAs yielded significant *F*-values ($P < 0.05$), treatment means were compared using Tukey's test (HSD). Before analysis, parasitism rates were arcsine-transformed, however non-transformed data are presented in the results section.

Results

Levels of parasitism of *H. hampei* by *P. coffea* were significantly affected by the age of the berries at the time of infestation, the position of the beetle inside the berries, and the age by position interaction (table 1). The time of the *P. coffea* releases after artificial infestation of berries with *H. hampei* did not affect parasitism levels. Similarly, the interaction between time of parasitoid release and age of the berries at the time of infestation had no effect on rates of parasitism. However, the interaction of time of parasitoid release and position of the beetle in the berries, as well as the three-way interaction between berry age at the time of infestation with *H. hampei*, parasitoid release time and the position of the beetle in the berry significantly affected rates of parasitism of *H. hampei* by *P. coffea* (table 1). Consequently, parasitism levels were compared at a given position of the beetle inside the berries and at a given time of the *P. coffea* releases across the different ages of the coffee berries at the time of infestation with *H. hampei*, as well as at a given age of the coffee berries at the time of infestation, for a given position of the beetle in the berries across the different times of *P. coffea* releases (table 2). In 90-day-old berries, the proportion of *H. hampei* parasitized in position A was significantly higher after one day compared to five and nine days after a *P. coffea* release. Independent of the time of the *P. coffea* releases, hardly any parasitism was detected in adults of *H. hampei* found in position A of 150-and 210-day-old berries. In 90-day-old berries, the level of parasitism in position B (60%) was significantly higher when the parasitoids were released one day compared to five and nine days after the artificial infestation with *H. hampei* (table 2). For the same position, significantly lower levels of parasitism were recorded in 150- and 210-day-old berries one day, and in 210-day-old berries also nine days after the parasitoid releases compared to 90-day-old berries. Significantly fewer *H. hampei* in position B (1.8%) were parasitized in 210-day-old berries nine days after the *P. coffea* releases compared to the two earlier parasitoid releases (table 2). For *H. hampei* in position C, highest levels of parasitism were recorded in 150- compared to 90- and 210-day-old berries independent of the *P. coffea* release dates (table 2). With increasing time between

Table 2. Parasitism of coffee berry borer (CBB) *Hypothenemus hampei* adults (%±SE) by *Phymastichus coffea* released one, five and nine days after host infestation in each position of penetration in 90-, 150- and 210-day old coffee berries.

Position of CBB in the berries*	Time of *P. coffea* release (days)	CBB found parasitized in each position (%±SE)		
		90 days	150 days	210 days
A	1	12.5±3.6aA	0.0±0.0bA	0.0±0.0bA
	5	0.0±0.0aB	0.3±0.3aA	0.0±0.0aA
	9	0.0±0.0aB	0.5±0.3aA	0.0±0.0aA
B	1	60.0±6.8aA	14.5±3.7bA	9.5±3.7bA
	5	19.0±2.4aB	14.3±1.9aA	10.8±4.7aA
	9	20.3±3.3aB	25.5±6.9aA	1.8±0.8bB
C	1	3.3±1.2cB	70.5±2.9aA	19.0±4.6bA
	5	34.0±4.6bA	66.5±2.9aA	16.5±4.1cA
	9	29.5±2.5bA	47.3±6.5aB	20.8±2.8bA
D	1	0.0±0.0bA	0.0±0.0bB	3.3±1.0aA
	5	0.0±0.0bA	0.0±0.0bB	4.5±1.7aA
	9	0.0±0.0bA	1.8±0.9bB	8.3±1.7aA

Means followed by the same small letter in each row and by the same capital letter in each column are not significantly different ($P > 0.05$, Tukey test).
*See text for definition of positions A, B, C and D.

artificial infestations with *H. hampei* and *P. coffea* releases, parasitism significantly decreased in 90- and 150-day-old berries. No or only very low parasitism levels were recorded in *H. hampei* found in position D (table 2).

Because of the significant three-way interaction between age × release × position (table 1) total levels of parasitism could not be compared statistically among age classes of coffee berries and/or parasitoid release time intervals. However, highest levels of total parasitism were recorded in 150-day-old berries, and one day after a *P. coffea* release also in 90-day-old coffee berries (table 3). Additionally, percentages of parasitism for a given age of the berries at the time of infestation with *H. hampei* and for a given *P. coffea* release were compared across the different positions of the beetle inside the coffee berries. In 90-day-old berries, significantly highest parasitism levels were recorded in position B one day after the *P. coffea* release and in position C five and nine days after the parasitoid releases (table 3). In 150-day-old berries, independent of the time of the *P. coffea* releases, significantly highest levels of parasitism were found in position C followed by position B. Similarly, in 210-day-old berries, significantly highest parasitism level was always recorded in *H. hampei* found in position C (table 3). For *H. hampei* in position D, low levels of parasitism were only

recorded in 150-day-old berries nine days after the *P. coffea* release and in 210-day-old coffee berries independent of the time of the release of the parasitoids (table 3).

Discussion

Levels of parasitism of *H. hampei* approached 85% following parasitoid releases, suggesting that *P. coffea* had a strong impact on its host under field conditions in Colombia. However, parasitism levels were significantly affected by the developmental stage of the coffee berries and by the position of the beetle inside the coffee berries at the time of the parasitoid releases. The speed of penetration of *H. hampei* in coffee berries depends on the physiological state of the berry, i.e. their dry matter content (Arcila *et al.*, 1993). The time between initial colonization of a coffee berry by a *H. hampei* female, i.e. positions A and B, and subsequent oviposition, i.e. position D, under field conditions in Colombia are 70 and 5 days, for 90- and 210-day-old berries, respectively (Ruiz, 1995). In the present study, the highest levels of parasitism were recorded in beetles found in position C of 150-day-old berries independent of the time of the parasitoid releases. At this time, parasitized adult *H. hampei* found in position C had just begun damaging the

Table 3. Parasitism of coffee berry borer (CBB) *Hypothenemus hampei* adults (%±SE) by *Phymastichus coffea* released after one, five and nine days of infestation in each position of penetration (A, B, C, D) in 90-, 150- and 210-day-old coffee berries.

Age of berries at infestation (days)	Time of *P. coffea* release (days)	Total parasitism (%±SE)	CBB found parasitized in each position* (%±SE)			
			A	B	C	D
90	1	75.5±6.8	12.5±3.6b	60.0±6.8a	3.3±1.2bc	0.0±0.0d
	5	53.0±3.8	0.0±0.0c	19.0±2.4b	34.0±4.6a	0.0±0.0c
	9	49.8±2.4	0.0±0.0c	20.3±3.3b	29.5±2.5a	0.0±0.0c
150	1	85.0±2.1	0.0±0.0c	14.5±3.7b	70.5±2.9a	0.0±0.0c
	5	81.0±2.1	0.3±0.3c	14.3±1.9b	66.5±2.9a	0.0±0.0c
	9	75.0±1.4	0.5±0.3c	25.5±6.9b	47.3±6.5a	1.8±0.9c
210	1	31.8±5.2	0.0±0.0c	9.5±3.7ab	19.0±4.6a	3.3±1.0bc
	5	31.8±4.8	0.0±0.0c	10.8±4.7ab	16.5±4.1a	4.5±1.7bc
	9	30.8±3.1	0.0±0.0c	1.8±0.8c	20.8±2.8a	8.3±1.7b

Means followed by the same letter within each row are not significantly different ($P > 0.05$, Tukey test).
*See text for definition of positions A, B, C and D.

endosperm and in no case were oviposition chambers observed. Female *H. hampei* stop ovipositing and their mobility is impaired after parasitization by *P. coffea* (Feldhege, 1992; Infante *et al.*, 1994). In 90-day-old berries when *P. coffea* was released one day after the artificial infestation with *H. hampei*, around 60% of the parasitized beetles were found in position B. Yet when the parasitoids were released five or nine days after infestation, highest levels of parasitism were recorded in *H. hampei* females in position C, suggesting that beetles originally attacked by *P. coffea* in position B thereafter penetrated further into the coffee berries. A similar behaviour has been observed in Ips typographus Linnaeus (Coleoptera: Scolytidae) and *Tomicobia seitneri* (Ruschka) (Hymenoptera: Pteromalidae), where parasitized beetles continued to bore into the bark (Sachtleben, 1952). Likewise, Feldhege (1992) observed that *H. hampei* parasitized by *P. coffea* continued boring into the berries for some days until they died. In laboratory studies, *P. coffea* females were unable to penetrate into coffee berries and attack *H. hampei* females in positions C or D (Borbón, 1989; Infante *et al.*, 1994; Lopez & Moore, 1998). Thus the high levels of parasitism recorded in 90- and 150-day-old berries might be due to the long time *H. hampei* were exposed to *P. coffea* while penetrating the exocarp. Once the berries start to mature and have acquired >20% dry matter content in the endosperm, *H. hampei* females bore deeper into the berries (Bergamin, 1943; Alonzo, 1984), and are there probably less at risk of an attack by *P. coffea*. This is supported by the low levels of parasitism in *H. hampei* in 210-day-old berries. Then parasitized beetles were predominantly found in position C, though parasitism never exceeded 21%. Moreover, less than 9% of the beetles found in position D were parasitized and only in berries older than 159 days. While constructing the galleries in the endosperm, female *H. hampei* often expose their abdomen for short periods outside the berry to remove the detritus (Bustillo *et al.*, 1998), and are then exposed to an attack by *P. coffea*. This might be one factor explaining the parasitism of *H. hampei* in position D in 210-day-old coffee berries.

The results of the present study showed that *P. coffea* is a promising biological control candidate for coffee berry borer although its parasitism potential decreased with the age of the coffee berries. Consequently, the decision of the release period of *P. coffea* in a coffee plantation should be based on the age of the berries which can be determined by recording the major blossoming period (Bustillo *et al.*, 1998), and also on long-term climatic data. Populations of *H. hampei* tend to remain inside coffee berries and reproduce at a higher rate during the drier periods, whereas reproduction decreases and migration and subsequent colonization of new berries increase during the rainy season (Baker *et al.*, 1994; Bustillo, 2002). Generally, *H. hampei* populations start colonizing coffee berries between 100 to 150 days after blossoming (Salazar *et al.*, 1993). Since *H. hampei* females are at this time mainly found in positions A and B, inoculative or augmentative releases of *P. coffea* should be carried out during this period. However, in older berries, >160 days after blossoming, most of the beetles have already penetrated too deep into the endosperm for *P. coffea*. Thus other biocontrol agents like the two larval–pupal cryptoparasitoids *C. stephanoderis* and *P. nasuta* should be released to complement *P. coffea*. Both larval–pupal parasitoids are capable of parasitizing *H. hampei* in positions C and D (Baker, 1999). However, in field studies so far only low levels of parasitism by these two

bethylids have been recorded in Mexico and Colombia (Baker, 1999; Damon, 2000).

The parasitoid–host ratio used in releases in this study was high and could be too costly to implement in an area-wide management programme. However, attempts are underway to improve the rearing of *H. hampei*, a crucial factor for the parasitoid production, through the development of an artificial diet (Portilla, 1999). Moreover, the first field releases of *P. coffea* were followed by the successful establishment of the parasitoid in Colombia (Baker, 1999; Aristizabal *et al.*, 2004) suggesting that this parasitoid can be used in classical biological control of the coffee berry borer. In summary, our data clearly underlines the great potential of *P. coffea* for biological control of the coffee berry borer in Colombia and other coffee growing countries of South and Central America.

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