



Where to sample? Ecological implications of sampling strata in determining abundance and impact of natural enemies of the coffee berry borer, *Hypothenemus hampei*

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ABSTRACT

Several parasitoids of African origin have been introduced to coffee producing areas of the Americas and Asia as biological control agents of the coffee berry borer (CBB) *Hypothenemus hampei* (Coleoptera: Curculionidae). These parasitoids have become established in the field but their effect on the CBB has been limited. A two-year field study in Western Kenya has found *Prorops nasuta* (Hymenoptera: Bethyilidae) to be the predominant parasitoid emerging from CBB-infested coffee berries collected on coffee trees or from the ground. *P. nasuta* comprises more than 75% of the total natural enemies collected. The density of *P. nasuta* was 90% higher in the berries collected from the ground than from the trees. Its hyperparasitoid, *Aphanogmus* sp. (Hymenoptera: Ceraphronidae), also emerged from both type of berries. Across the two seasons, the average *P. nasuta* density per berry was 18–35 times higher than that of *Aphanogmus* sp. Throughout the two years sampled, significantly higher numbers of *P. nasuta* and *Aphanogmus* sp. occurred between February and March, which coincides with the beginning of the rainy season. Higher numbers of live CBB females were recorded in berries collected from the trees. Nevertheless, mortality of adult CBB was considerably higher from January to March and started to decrease from April onwards. The possibly negative effects of cultural control practices in Latin America which include the removal of berries fallen to the ground on biological control of CBB are discussed, and the use of screened collection devices for these berries which would permit the release of parasitoids but prevent escape of the pest is proposed.

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1. Introduction

Endemic to Central Africa, the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae) is the most devastating insect pest of commercial coffee worldwide (Le Pelley, 1973; Damon, 2000; Jaramillo et al., 2006). Female insects bore galleries in the berry where they oviposit up to 200 eggs, causing qualitative and quantitative losses through larval feeding of the endosperm. The cryptic nature of CBB inside the berry, combined with a skewed sex ratio favoring females (10:1) and sibling mating inside the berry makes this insect quite difficult to control. Reported infestation levels of the pest can be extremely high, e.g., 60% in Colombia, 58–85% in Jamaica, 50–90% in Malaysia, and 60% in Mexico (Vega, 2004). Due to the insects' concealed nature, biological

control is the most promising management option against *H. hampei*.

Previous explorations for natural enemies of CBB in Africa have revealed the presence of various parasitoids, including the braconid *Heterospilus coffeicola* Schmiedeknecht, which was first reported by Hargreaves (1926) in Uganda. However, so far no viable rearing protocols have been developed for this wasp, limiting its use in biological control programs (Murphy et al., 2001). In addition, two bethyloid parasitoids of CBB have been discovered: *Cephalonomia stephanoderis* Betrem and *Prorops nasuta* Waterston originating from Ivory Coast (Ticheler, 1961) and Uganda (Hempel, 1934), respectively. These bethyloids are larval–pupal ectoparasitoids of CBB and usually prey on females and eggs (Pérez-Lachaud et al., 2002; Infante et al., 2005). A fourth parasitoid species, the eulophid *Phymastichus coffea* LaSalle was recorded first in Togo in 1987 (Borbón-Martinez, 1989).

Cephalonomia stephanoderis and *P. nasuta* have been introduced to coffee growing areas in the Americas (Barrera et al., 1990; Baker, 1999) and to India (Duque and Baker, 2003). In the Americas they

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are presently being used in an integrated pest management (IPM) program combined with entomopathogenic fungi like *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), trapping, and cultural control. Although, *C. stephanoderis* and *P. nasuta* became established in the all release countries in the Americas, levels of parasitism in *H. hampei* field populations rarely exceed 5% (Quintero et al., 1998; Baker, 1999; Infante et al., 2001).

The cultural control component of the CBB IPM program in the Americas involves the complete removal of all ripe and over-ripe berries after the harvest and during the inter-harvest period thus reducing vital sources of re-infestations. Rigorous collection of berries from the trees and from the ground, termed 'Re-Re' in Spanish for 'Recolección' and 'Repase' (harvesting of berries and immediately thorough re-collection of remaining berries in the same field), can substantially reduce infestations of the pest in the field for two reasons: (i) immigrating CBB females from populations outside the field will not find suitable berries for oviposition, thus breaking the infestation cycle of the pest and (ii) dry berries harboring CBBs are removed, thereby reducing the *H. hampei* source population of the field/plantation (Bustillo et al., 1998). It is estimated that 89% of the total CBB management costs go to personnel due to this laborious cultural control practice (Duque and Baker, 2003). In spite of its cost, an analysis of the different CBB IPM options in Colombia concluded that cultural control is the most important and widely used component (Aristizabal et al., 2002; Benavides et al., 2002).

The objective of this study was to evaluate the seasonal ecology and dynamics of CBB bethylid parasitoids in a coffee growing area of Kenya, over two fruiting periods, in coffee berries collected from the trees and in coffee berries that had fallen to the ground.

2. Materials and methods

Parasitoids associated with CBB were sampled from October 2006 to September 2008 in the Kisii area of Western Kenya (00° 25' S, 34° 28' E, 1510 m above sea level [masl]). A plantation consisting of 2000 trees of *Coffea arabica* L. (var. Ruiru 11) was selected for the study. Climatic data, i.e. monthly values of mean, max and min temperature, relative humidity (RH) and precipitation were obtained from the Kenyan Agriculture Research Institute (KARI).

Samples of CBB-infested coffee berries both from the trees and the ground, i.e. the litter strata, were collected at 2–3 weeks intervals for 2006 and 2007 and weekly during 2008. Between 100 and 150 trees were sampled randomly at each evaluation date, collecting as many CBB-infested berries on the branches and from the ground as possible.

The coffee berries were surface sterilized in the laboratory to reduce fungal contamination during the period in which berries were to be sampled for parasitoid emergence. The sterilization procedure (Pérez et al., 2005) consists of washing the berries with detergent for 15 min, rinsing with tap water, then dipping in a 2% sodium hypochlorite solution for 10 min, rinsing again with sterile distilled water, thereafter soaking in a 2% potassium sorbate solution and finally rinsing with sterile distilled water. Subsequently the coffee berries were allowed to dry at room temperature. After surface sterilization, the berries were placed in square plastic containers (40 × 40 × 20 cm) with perforated lids (55 mm dia), covered with mesh to avoid the escape of the parasitoids. Each container was filled with a 3 cm layer of a mixture of plaster of Paris and activated charcoal to maintain the humidity and prevent the desiccation of the berries (Jaramillo et al., 2009), thus allowing to record the emergence of the natural enemies for periods of up to 90 days after each sampling date. The interior of the containers was watered every 3 days to maintain the humidity inside the container; containers were kept at room temperature (ca. 25 ± 2 °C, 70% ± 5% RH and L12:D12 photoperiod). The berries were cleaned

every 2 days to remove the CBB frass. Emergence of parasitoids was assessed daily, and emerged parasitoids were recorded and individually transferred to 0.5 ml Eppendorf® tubes containing 95% ethanol. During 2008, in addition to recording the emergence of parasitoids a sub-sample of 100 berries was taken from each sampling date once parasitoids ceased to emerge; these berries were then dissected under the stereomicroscope (10×), in search for natural enemies that had not emerged from the coffee berries. Specimens were shipped to and then identified at the Systematic Entomology Laboratory, United States Department of Agriculture, Agricultural Research Service (USDA-ARS), in Beltsville, Maryland, USA.

2.1. Statistical analyses

Data on abundance and emergence of parasitoids are presented separately for berries collected on the ground and in the trees. Shannon's diversity index (Shannon, 1948) was calculated individually for parasitoids emerging from berries from the tree and ground strata for all sampling months, using the following formula:

$$(1) H' = - \sum_{i=1}^s p_i \ln p_i$$

where S is the number of species, and p_i is the relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community. Shannon diversity index increases as the number of different species increases and/or the proportional distribution of individuals among species becomes more equitable. For a given number of species, the maximum value of the Shannon index is reached when there are similar proportions of all species.

A covariance analysis was performed, using the GLM procedure of SAS (SAS Institute Inc., 1999) to investigate the effects of sampling months and coffee berry strata on the abundance (average insect per berry per sampling month) of *P. nasuta* and *Aphanogmus* sp. (Hymenoptera: Ceraphronidae), where the total number of berries collected was used as a co-variable. The estimates of parameters that contribute to the abundance of the parasitoid and its hyperparasitoid were calculated. Averages of *P. nasuta* and *Aphanogmus* sp. per berry per month were log transformed prior to analysis. For each parameter that contributed to the abundance, the t -value was calculated and the significant level was set at 0.05.

Dissection data were analyzed by means of analysis of variance ANOVA, using the general linear model (PROC GLM) of SAS (SAS Institute Inc., 1999). In case the ANOVAs yielded significant F-values, means were compared using Tukey's test (HSD).

Percentage parasitism (Van Driesche, 1983) was calculated separately for the samples for the tree and ground berry samples using the following formula:

$$\% \text{ Parasitism} = (\text{emergence of parasitoids per 100 berries} + \text{parasitoid immature stages and adults found during dissections per 100 berries}) / (\text{decapitated CBB females} + \text{parasitoid cocoons}).$$

3. Results

3.1. Parasitoid species complex

Emergence of parasitoids was recorded from a total of 32,780 berries collected from the trees and 36,729 berries from the litter (ground) strata, between October 2006 and September 2008. An

additional 3842 berries sampled in 2008 were dissected for CBB life stages and natural enemies. In total 333 parasitoid/hyperparasitoid specimens were collected from berries harvested from the trees and 10,409 emerged from the ground strata samples. All of these parasitoids/hyperparasitoids are considered to be associated with CBB (Table 1). Across sampling periods, eight species were collected (four species from the trees vs. seven from the ground strata) resulting in a Shannon index of 0.73 for tree coffee berries and 0.6 for the ground strata.

For coffee berries collected from the trees, *P. nasuta* was the dominant parasitoid species with 71.5% of the total parasitoid emergence, followed by unidentified parasitoid sample #57 (24.0%), *Tapinoma* sp. (Hymenoptera: Formicidae: Dolichoderinae) sample #111 (2.7%), *Aphanogmus* sp. (1.2%), *P. coffea* (0.3%) and *C. stephanoderis* (0.3%).

From the coffee berries that were collected on the ground, the most dominant parasitoid was again *P. nasuta* accounting for 82.2% of the total emergence, followed by its hyperparasitoid *Aphanogmus* sp. (12.7%), unidentified parasitoid sample #57 (4.4%), unidentified parasitoid sample #54 (0.1%), *P. coffea* (0.1%), *P. near schedli* (0.1%), *C. stephanoderis* (0.02%) and *Goniozus* sp. (Hymenoptera: Bethyilidae) (0.06%).

3.2. Relative abundance of CBB parasitoid species over the sampling periods

Fig. 1 shows the abundance of the CBB parasitoid complex over time for the trees and ground strata. The dominant species were *P. nasuta* and its hyperparasitoid *Aphanogmus* sp. For berries collected from the ground, considerably higher numbers of species were recorded during the first months of 2007 compared to the same period in 2008. *Tapinoma* sp. was only collected from tree berries between June and July 2007. *Aphanogmus* sp. was collected in different months on ground berries and was only recorded one time in August 2007 on tree berries.

Table 1

Most abundant species recovered from coffee berries collected on the ground and from coffee trees.

Species	Habit	Place of collection	N
<i>Hymenoptera</i>			
<i>Bethyilidae</i>			
<i>Cephalonomia stephanoderis</i>	Primary CBB parasitoid (Ticheler, 1961)	Ground Tree	2 1
<i>Prorops nasuta</i>	Primary CBB parasitoid (Hargreaves, 1926)	Ground Tree	8180 240
<i>Goniozus</i> sp.	Possible new CBB parasitoid	Ground Tree	6 0
<i>Ceraphronidae</i>			
<i>Aphanogmus</i> sp.	<i>P. nasuta</i> hyperparasitoid (Jaramillo and Vega, 2009)	Ground Tree	1342 4
<i>Eulophidae</i>			
<i>Phymastichus coffea</i>	Primary CBB parasitoid (Borbon, 1989)	Ground Tree	16 1
<i>Formicidae</i>			
<i>Tapinoma</i> sp.	Possible CBB predator	Ground Tree	0 9
<i>Pteromalidae</i>			
<i>Pachycrepoides near schedli</i>	Possible CBB parasitoid (OILB, 1971)	Ground Tree	13 0
<i>Others</i>			
Sample 54	Under identification	Ground Tree	12 0
Sample 57	Under identification	Ground Tree	430 0

3.3. Dynamics of *P. nasuta* and its hyperparasitoid *Aphanogmus* sp. on tree and ground berries

The analysis of covariance using the total berries as covariate indicated that the abundance of *P. nasuta* and its hyperparasitoid *Aphanogmus* sp. varied significantly with coffee berry strata ($F_{1, 97} = 40.28$, $P < 0.0001$ and $F_{1, 97} = 24.94$, $P < 0.0001$ for *P. nasuta* and *Aphanogmus* sp., respectively). Their occurrence was significantly higher on ground compared to tree berries (Table 2). Likewise, time of the year had a significant impact on numbers of *P. nasuta* and *Aphanogmus* sp. ($F_{1, 97} = 6.03$, $P < 0.0001$ and $F_{1, 97} = 5.82$, $P < 0.0001$ for *P. nasuta* and *Aphanogmus* sp., respectively). Across the two sampling years and coffee berry strata, significantly higher numbers of *P. nasuta* and *Aphanogmus* sp. occurred between February and March (Table 2).

3.3.1. Ground

During the three sampling months in 2006, low numbers of *P. nasuta* and *Aphanogmus* sp. were recorded (Fig. 2). The average density per infested berry increased in early January 2007 for *P. nasuta*, and for *Aphanogmus* sp. peaked during February and March of the same year (Fig. 2). A second peak was observed in February 2008 for both *P. nasuta* and *Aphanogmus* sp. Across the seasons, the average *P. nasuta* density per berry was 18–35 times higher than that of *Aphanogmus* sp. (Fig. 2).

3.3.2. Tree

Densities of both *P. nasuta* and *Aphanogmus* sp. were relatively lower in berries sampled from the trees compared to the ground stratum (< 0.08 insect per berry) (Fig. 3). A first peak for *P. nasuta* was observed in May 2007, a second in December 2007 and the last one in March 2008.

3.4. Dissection of coffee berries

Dissections of the coffee berries were carried out only in 2008 after determining a trend in the emergence of the parasitoids. Tree or ground strata and the month of collection had a significant effect on the numbers of CBB immature stages ($F_{1, 3662} = 10.21$, $P = 0.0014$) ($F_{7, 3662} = 39.5$, $P < 0.0001$), total numbers of CBB females ($F_{1, 3662} = 14.8$, $P < 0.0001$) ($F_{7, 3662} = 38.7$, $P < 0.0001$), females found live ($F_{1, 3662} = 30.0$, $P < 0.0001$) ($F_{7, 3662} = 11.4$, $P < 0.0001$), dead ($F_{1, 3662} = 138.7$, $P < 0.0001$) ($F_{7, 3662} = 53.7$, $P < 0.0001$) and CBB female mortality ($F_{1, 3662} = 347.5$, $P < 0.0001$) ($F_{7, 3662} = 79.1$, $P < 0.0001$). Likewise the interaction sampling strata by month of collection was always significant except in the case of CBB immature stages ($F_{7, 3662} = 0.52$, $P = 0.8168$) (Figs. 4–6).

In general, a high percentage of berries were attacked by CBB and the infestation ranged from 60–91% and 44–84% for ground and tree berries, respectively. However, the total numbers of CBB females per berry was low, ranging from 2.9 in February to 9.9 in March for ground berries (Fig. 5a) and between 2.4 in May and 7.6 in March. CBB females that were found alive during the dissections were higher in tree compared to ground berries (Fig. 5b). The number of immature stages started to increase from February reaching the maximum number in June (Fig. 4). The mortality of CBB was considerably higher during January, February and March and started to decrease from April onwards (Fig. 6). This reduction of CBB mortality coincided with a decrease in numbers of *P. nasuta* emerging from the samples (Figs. 2 and 3).

Hardly any life stages of *P. nasuta* were found in tree berries. However, the sampling strata and month of collection of the coffee berries had an effect on the numbers for cocoons ($F_{4, 1351} = 16.6$, $P < 0.0001$), for *P. nasuta* live ($F_{4, 1351} = 12.9$, $P < 0.0001$), and for *P. nasuta* dead ($F_{4, 1351} = 26.4$, $P < 0.0001$). In ground berries, be-

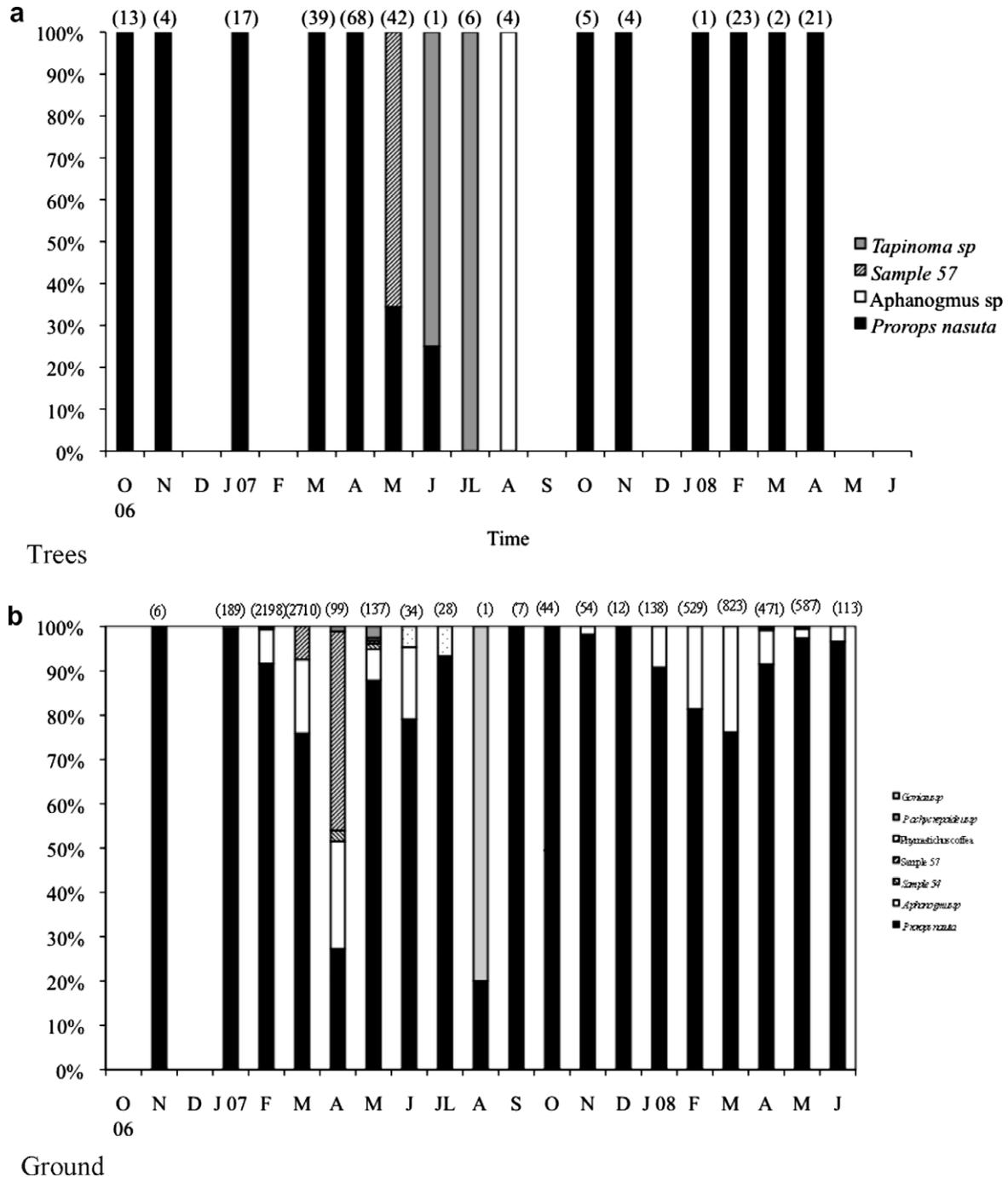


Fig. 1. Temporal dynamics of the *Hypothenemus hampei* parasitoid complex recorded from coffee berries collected on coffee trees or from, (b) the ground. Numbers in parenthesis indicate the number of *Prorops nasuta* that emerged each month for ground berries and during July and August in (a) (emergence from the trees) indicates numbers of *Tapinoma* sp. and *Aphanogmus* sp., respectively.

tween 0.08 and 0.66 dead and alive adult *P. nasuta* and 0.26 to 1.84 cocoons of the parasitoid were found inside a single berry. Moreover, no *Aphanogmus* sp. individuals were detected when dissecting berries collected on the ground and in the trees.

3.5. Percentage of parasitism

In ground berries, parasitism of CBB by *P. nasuta* was 17.8%, 49.1%, 21.2%, 31.0%, and 47.1% for the months of January, February, March, April and May 2008, respectively. Only four *P. nasuta* adults and no cocoons were found after dissecting the berries collected

from trees; therefore levels of parasitism could not be estimated for this stratum.

4. Discussion

Two years of intensive sampling in a coffee plantation in Western Kenya revealed a surprisingly low diversity of CBB parasitoids, as illustrated by the low Shannon index values. Apart from the bethylid parasitoid *P. nasuta* and its ceraphronid hyperparasitoid *Aphanogmus* sp. (Jaramillo and Vega, 2009), all other known parasitoids of *H. hampei* were either absent or recorded in very low

Table 2

Covariance analysis of the effect of sampling months and coffee berry sampling strata on the abundance of *Prorops nasuta* and its hyperparasitoid *Aphanogmus* sp.

Variables	Log (mean <i>Prorops nasuta</i> per berry)				Log (mean <i>Aphanogmus</i> sp. per berry)			
	Estimate	SE	t-Value	P-Value	Estimate	SE	t-Value	P-Value
<i>Months</i>								
January	0.0425	0.0284	1.5000	0.1378	0.0061	0.0073	0.8300	0.4083
February	0.1249	0.0293	4.2700	0.0001	0.0237	0.0075	3.1300	0.0023
March	0.1061	0.0287	3.7000	0.0004	0.0320	0.0074	4.3200	0.0001
April	0.0222	0.0333	0.6700	0.5066	0.0093	0.0086	1.0800	0.2814
May	0.0166	0.0316	0.5300	0.6002	0.0007	0.0081	0.0900	0.9306
June	0.0016	0.0304	0.0500	0.9570	0.0007	0.0079	0.0900	0.9249
July	0.0056	0.0292	0.1900	0.8492	0.0000	0.0075	0.0000	0.9994
August	-0.0020	0.0298	0.0700	0.9457	0.0000	0.0077	0.0000	0.9962
September	0.0000	-	-	-	0.0000	-	-	-
October	-0.0026	0.0272	0.1000	0.9238	-0.0007	0.0070	0.0900	0.9250
November	-0.0001	0.0297	0.0000	0.9986	0.0001	0.0077	0.0100	0.9932
December	-0.0016	0.0294	0.0500	0.9570	0.0000	0.0076	0.0000	0.9977
Ground strata	0.0706	0.0111	6.3400	0.0001	0.0143	0.0029	4.9900	0.0001
Tree strata	0.0000	-	-	-	0.0000	-	-	-
Total berries	0.0000	0.0000	0.0900	0.9291	0.0000	0.0000	0.0200	0.9808
Intercept	-0.0264	0.0217	1.2200	0.2271	-0.0072	0.0056	1.2800	0.2023

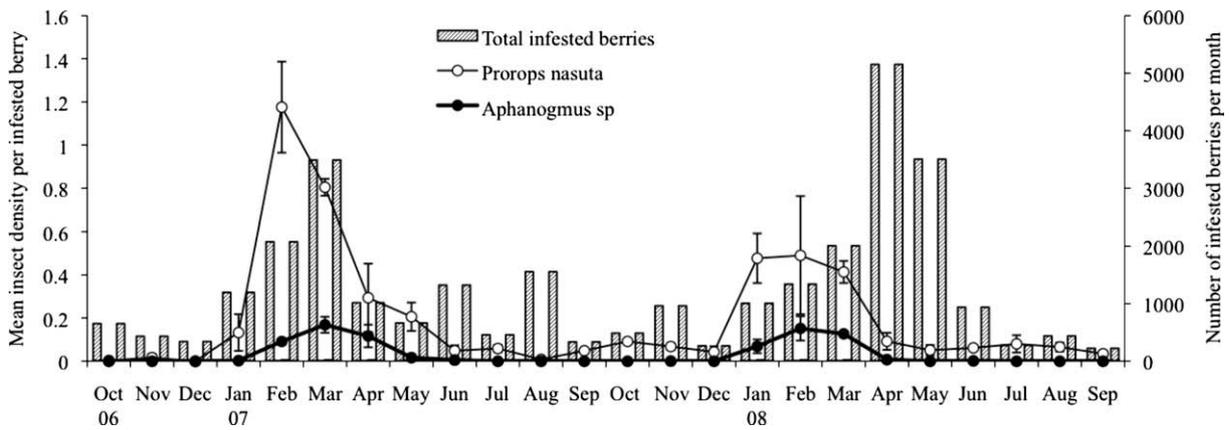


Fig. 2. Average numbers of *Prorops nasuta*, its hyperparasitoid *Aphanogmus* sp. and the total number of infested coffee berries collected per month from the ground strata.

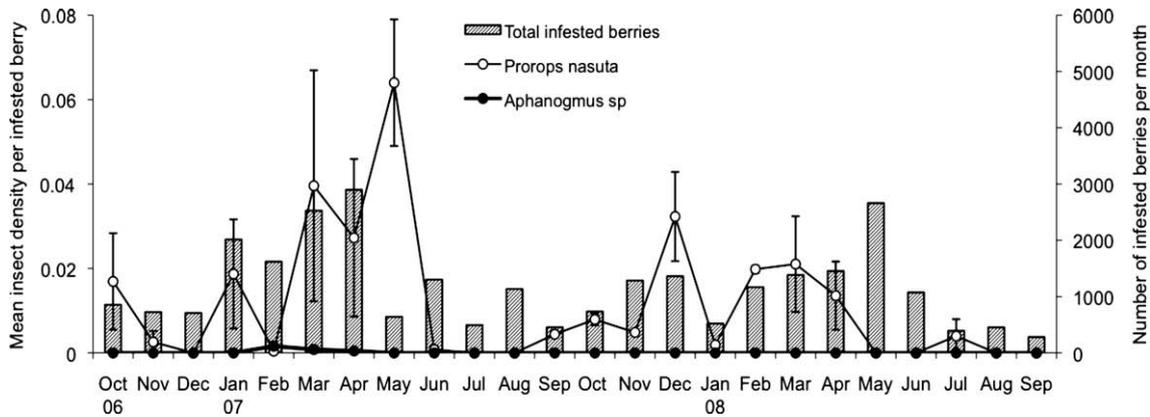


Fig. 3. Average numbers of *Prorops nasuta*, its hyperparasitoid *Aphanogmus* sp. and the total number of infested coffee berries collected per month from the tree strata.

numbers. For instance the eulophid *P. coffea* was virtually nonexistent in our sampling area during the two years of the study. Yet the original collections of the wasps that were subsequently introduced to Colombia as part of a classical biological control program for CBB were carried out exactly in the same area of Western Kenya where all data for this study was gathered (Baker, 1999). It was also striking that the braconid *H. coffeicola* was never found during our two years of sampling. In a similar study in neighboring Ugan-

da, Hargreaves (1926) reported *H. coffeicola* and *P. nasuta* as the two predominant natural enemies of the CBB.

One might argue that the low diversity of natural enemies recorded in our study is due to the fact that we restricted our sampling to one coffee plantation only. Yet the focus of this research was to elucidate the seasonal dynamics of natural enemies of CBB, and hence we sampled throughout two entire fruiting seasons, i.e. from blossoming to harvest. In contrast pre-

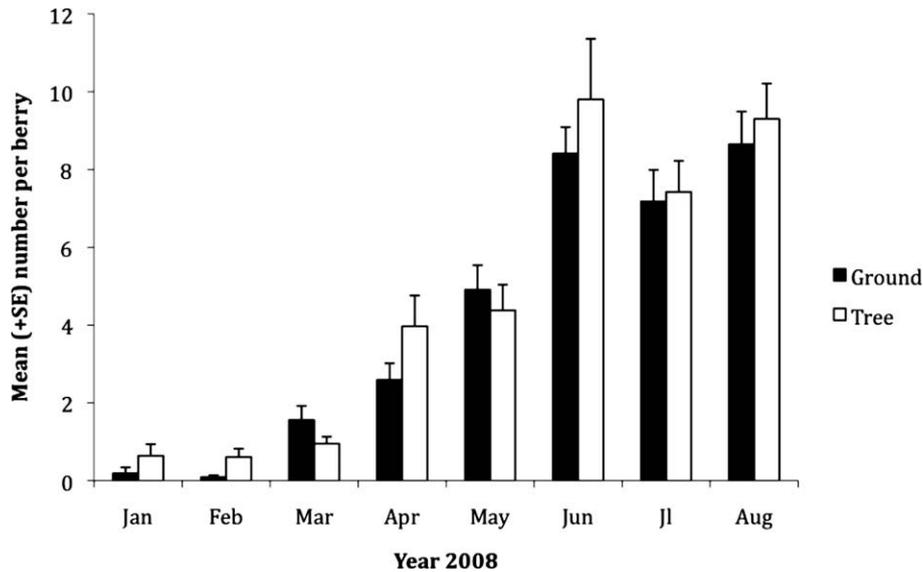


Fig. 4. Mean (+SE) numbers of immature stages of *Hypothenemus hampei* inside coffee berries collected on coffee trees or on the ground over time.

vious studies followed more a survey approach, often resulting in only snapshot-like information on the diversity of natural enemies of CBB. Thus despite the fact that we sampled only in one plantation we were able to record with *Aphanogmus* sp. a previously unknown hyperparasitoid of *P. nasuta* (Jaramillo and Vega, 2009), and discover a new specialized predator of CBB (Jaramillo, unpublished results).

Vega et al. (1999) recorded *C. stephanoderis* as the most prevalent CBB parasitoid in Togo. Out of the 10,342 CBB parasitoids identified in the present study only two specimens of *C. stephanoderis* were obtained. According to Ticheler (1961) and Hargreaves (1935), *C. stephanoderis* and *P. nasuta* are the dominant parasitoids of *H. hampei* in West and East Africa, respectively.

We recorded one major peak in emergence (February to May) for *P. nasuta* in our study area; this is in agreement with observations by Hargreaves (1926) in Uganda, where one peak was also reported in this country, although it occurred later in the year. *Aphanogmus* sp., a hyperparasitoid of *P. nasuta*, started to appear approximately one month later. Around 10% of the *P. nasuta* cocoons were hyperparasitized, and between two and three *Aphanogmus* sp. adults on average emerged from the host cocoons (Jaramillo and Vega, 2009).

An additional explanation for the low biodiversity of CBB parasitoids in our study sites might be the type of coffee plantation we sampled. Despite the organic production system used, the plantation was not shaded and was surrounded by other crops. Intensification of the coffee system has been reported to lead to low insect biodiversity (Perfecto et al., 2003; Richter et al., 2007).

Why has *P. nasuta*, in spite of being the key natural enemy of CBB in East Africa, as indicated by our data for Western Kenya and by Hargreaves (1926) for Uganda, been such an ineffective biological control agent in the Americas? A recent study from Colombia reported 73% establishment of *P. nasuta* in coffee farms but parasitism levels of only 0.25–19.5% (Maldonado, 2007). Similarly, reports from Infante (1998) in Mexico suggest that *P. nasuta* is only able to maintain high populations in the field if there are multiple releases. Previous attempts to resolve this riddle focused on the potentially negative interactions between *C. stephanoderis*, *P. nasuta* and *C. hyalinipennis* Ashmead, the latter being indigenous to the new world (Pérez-Lachaud et al., 2002, 2004; Batchelor et al., 2005, 2006). However, Batchelor et al.

(2006) has recently suggested that *P. nasuta* should be the most effective biological control agent of CBB due to its comparatively superior emergence rate and female offspring production. They also concluded that the failure of *P. nasuta* as a biological control agent in Mexico is hence not likely to be due to competitive interactions with the other bethylids.

In this study, out of the 8893 individual *P. nasuta* collected, only 2.7% emerged from berries picked from the trees, whereas the remaining 97.3% originated from berries that were collected on the ground. What is the importance of these findings for the control of CBB in the Americas? Presently, the most successful and widely adopted non-chemical control strategy against CBB in several Latin American countries promotes the complete removal and subsequent processing of all CBB-infested coffee berries from the trees as well as those that have fallen to the ground (Aristizabal et al., 2002), as originally proposed by Bergamin (1944b). Berries harboring the pest and which fell to the ground are a very important source for the re-infestation of next season's coffee (Baker, 1999; Bernal et al., 1999; Bustillo et al., 1999) as based on the fact that the CBB continues to reproduce and develop in these fallen berries (Bergamin, 1944a; Salazar et al., 1993). Our data from Western Kenya clearly shows that the coffee berries on the ground are not only the main reservoir of the beetles but also of its predominant parasitoid in East Africa, *P. nasuta*. We therefore hypothesize that the cultural control practice of removing infested coffee berries from the field may greatly affect the performance of *P. nasuta*.

As a parasitoid of immature stages of CBB, most of the life cycle of *P. nasuta* occurs within the coffee berries (Hargreaves, 1935; Abraham et al., 1990). In addition, the parasitoid generally attacks CBB when the coffee is close to harvesting (Hargreaves, 1935), and these nearly ripe berries are the main targets of cultural control of *H. hampei*.

There is ample evidence of positive effects of cultural control on biological control agents in the literature (e.g. Landis et al., 2000; Jonsson et al., 2008). However, negative interactions have received considerably less attention (van Emden and Service, 2004). For instance ploughing can negatively influence biological control of the sugar-beet weevil *Bothynoderes punctiventris* Germ (Coleoptera: Curculionidae) (van den Bosch and Telford, 1964), and it has been shown that coffee pruning affects natural enemies of the Antestia bug *Antestiopsis orbitalis* Westwood (H.F.

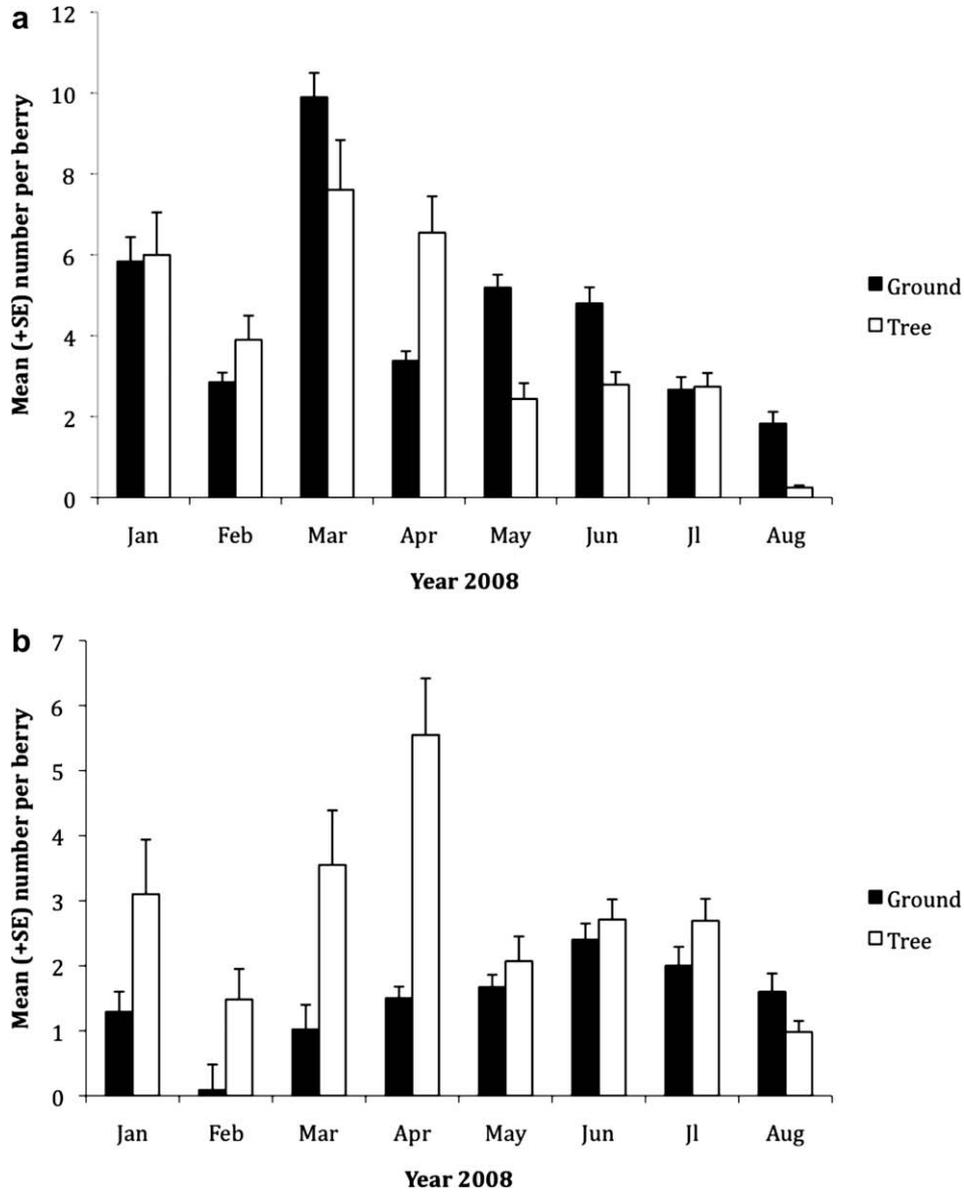


Fig. 5. Mean (+SE) numbers of (a) total *Hypothenemus hampei* females, and (b) *Hypothenemus hampei* live females inside coffee berries collected on coffee trees or on the ground over time.

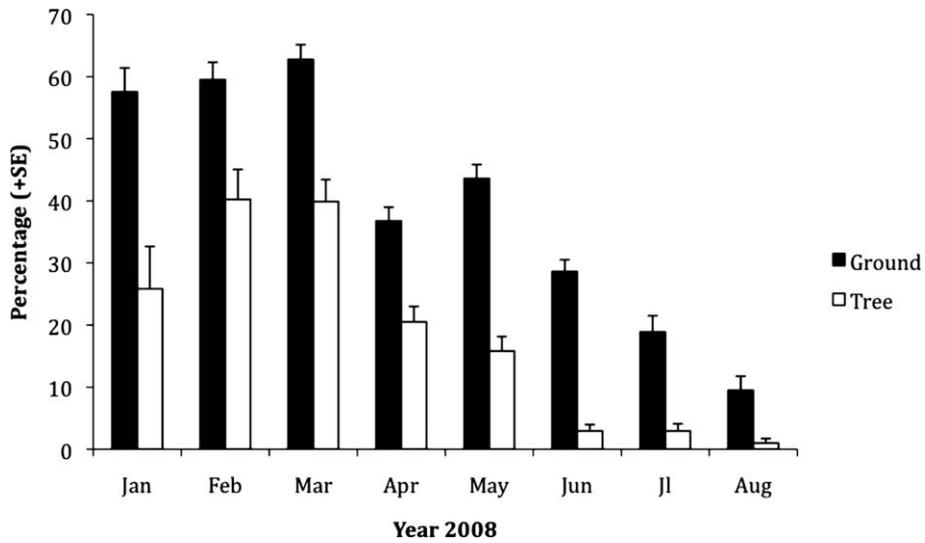


Fig. 6. Percent *Hypothenemus hampei* adult mortality in coffee berries collected on coffee trees or on the ground over time.

van Emden, pers. comm.). Yet, a strikingly similar example to CBB and *P. nasuta* has been reported in fruit flies: Purcell et al. (1994) found that the braconid *Diachasmimorpha longicaudata* (Ashmead), a parasitoid of the Oriental fruit fly *Bactrocera dorsalis* (Hendel), attacks its hosts in guava plantations primarily in fruits that have fallen to the ground; therefore orchard sanitation seriously affects parasitism rates. An ingenious solution to overcome this problem is a screened-enclosure called the augmentorium (Klungness et al., 2005), in which all infested fruits collected from the field are placed. The screen material used for its construction prevents the escape of tephritid flies emerging from infested fruit, but allows the escape of parasitoid wasps, thus minimizing the negative effects of crop sanitation on natural enemies (Jang et al., 2007). The use of the augmentorium has been shown to be highly efficient in reducing fruit fly populations in the field (Klungness et al., 2005).

Damon and Valle (2002) have reported that the efficacy of *C. stephanoderis* in Mexico was five times higher when coffee berries containing the wasps were released in the field, as compared to direct release of adult wasps. Thus, we believe that a structure similar to the augmentorium should be tested in coffee plantations in the Americas to harness the full biological control potential of *P. nasuta*. It seems likely that such an approach will result in increased parasitism levels by *P. nasuta*, and lower yield losses due to CBB infestation.

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References

- Abraham, Y.J., Moore, D., Godwin, G., 1990. Rearing and aspects of biology of *Cephalonomia stephanoderis* and *Prorops nasuta* (Hymenoptera: Bethylinidae) parasitoid of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Bulletin of Entomological Research* 80, 121–128.
- Aristizabal, L.F., Salazar, H.M., Mejía, C.G., 2002. Changes in the adoption of the components of coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae) integrated management, through participative methodologies. *Revista Colombiana de Entomología* 28, 153–160.
- Baker, P.S., 1999. The Coffee Berry Borer in Colombia. DFID-CENICAFE-CABI-BIOSCIENCE, Chinchiná, Colombia.
- Barrera, J.F., Baker, P.S., Valenzuela, J.E., Schwarz, A., 1990. Introducción de dos especies de parasitoides Africanos a México para el control biológico de la broca del café *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). *Folia Entomológica Mexicana* 79, 245–247.
- Batchelor, T.P., Hardy, I.C.W., Barrera, J.F., 2006. Interactions among bethylid parasitoid species attacking the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Biological Control* 36, 106–118.
- Batchelor, T.P., Hardy, I.C.W., Pérez-Lachaud, G., 2005. Insect gladiators II: competitive interactions within and between bethylid parasitoids species of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae). *Biological Control* 32, 194–202.
- Benavides, P., Bustillo, A., Montoya, E.C., Cardenas, R., Mejía, C., 2002. Participation of cultural, chemical and biological control in the management of the coffee berry borer. *Revista Colombiana de Entomología* 28, 161–165.
- Bergamin, J., 1944a. Sombreamento e broca. *Revista do Departamento Nacional do Café* 12, 1009–1014.
- Bergamin, J., 1944b. A broca do café no Brasil. *Boletim da Superintendencia dos Serviços do Café* (São Paulo) 33, 21–22.
- Bernal, M.G., Bustillo, A.E., Chavez, B., Benavides, P., 1999. Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on *Hypothenemus hampei* (Coleoptera: Scolytidae) populations emerging from fallen coffee fruits in the soil. *Revista Colombiana de Entomología* 25, 11–16.
- Borbón-Martínez, M.O., 1989. Bio-écologie d'un ravageur des baies de caféier *Hypothenemus hampei* Ferr. (Coleoptera: Scolytidae) et de ses parasitoides au Togo. PhD thesis, University of Toulouse, France.
- Bustillo, A.E., Bernal, M.G., Benavides, P., Chavez, B., 1999. Dynamics of *Beauveria bassiana* and *Metarhizium anisopliae* infecting *Hypothenemus hampei* (Coleoptera: Scolytidae) populations emerging from fallen coffee berries. *Florida Entomologist* 82, 491–498.
- Bustillo, A.E., Cardenas, R., Villalba, D., Benavides, P., Orozco, J., Posada, F.J., 1998. Manejo integrado de la broca del café *Hypothenemus hampei* (Ferrari) en Colombia. CENICAFÉ, Chinchiná, Colombia.
- Damon, A., 2000. A review of the biology and control of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae). *Bulletin of Entomological Research* 90, 453–465.
- Damon, A., Valle, J., 2002. Comparison of two release techniques of the use of *Cephalonomia stephanoderis* (Hymenoptera: Bethylinidae) to control the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae) in Soconuco, South Eastern Mexico. *Biological Control* 24, 117–127.
- Duque, O.H., Baker, P.S., 2003. Devouring Profit: The Socio-economics of Coffee Berry Borer IPM. The Commodities Press, CABI/CENICAFE.
- Hargreaves, H., 1926. Notes on the coffee berry borer (*Stephanoderes hampei*, Ferr.) in Uganda. *Uganda Bulletin of Entomological Research* 16, 347–354.
- Hargreaves, H., 1935. *Stephanoderis hampei* Ferr., coffee berry borer, in Uganda. *The East African Agricultural Journal* 1, 218–224.
- Hempel, A.A., 1934. *Prorops nasuta* Waterston no Brasil. *Arquivos do Instituto Biológico* 5, 197–212.
- Infante, F., 1998. Biological control of *Hypothenemus hampei* (Coleoptera: Scolytidae) in Mexico, using the parasitoid *Prorops nasuta* (Hymenoptera: Bethylinidae). PhD thesis. Imperial College, UK, p. 173.
- Infante, F., Mumford, J., Mendez, I., 2001. Non-recovery of *Prorops nasuta* (Hymenoptera: Bethylinidae), an imported parasitoid of the coffee berry borer (Coleoptera: Scolytidae) in Mexico. *Southwestern Entomologist* 26, 159–163.
- Infante, F., Mumford, J., Baker, P., 2005. Life history studies of *Prorops nasuta*, a parasitoid of the coffee berry borer. *BioControl* 50, 259–270.
- Jang, E.B., Klungness, L.M., Mcquate, G.T., 2007. Extension of the use of Augmentoria for sanitation in a cropping system susceptible to the alien tephritid fruit flies (Diptera: Tephritidae) in Hawaii. *Journal of Applied Science and Environmental Management* 11, 239–248.
- Jaramillo, J., Borgemeister, C., Baker, P.S., 2006. Coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae): searching for sustainable control strategies. *Bulletin of Entomological Research* 96, 223–233.
- Jaramillo, J., Chabi-Olaye, A., Poehling, H.M., Kamonjo, Ch., Borgemeister, C., 2009. Development of a new laboratory production technique for coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae), using fresh coffee berries. *Entomologia, Experimentalis et Applicata*, in press.
- Jaramillo, J., Vega, F.E., 2009. *Aphanogmus* sp. (Hymenoptera: Ceraphronidae): a hyperparasitoid of the coffee berry borer parasitoid *Prorops nasuta* Waterston (Hymenoptera: Bethylinidae) in Kenya. *Biocontrol Science and Technology*, in press.
- Jonsson, M., Wratten, S.D., Landis, D.A., Gurr, G.M., 2008. Recent advances in conservation biological control of arthropods by arthropods. *Biological Control* 45, 172–175.
- Klungness, L.M., Jang, E.B., Mau, R.F.L., Vargas, R.I., Sugano, J.S., Fujitani, E., 2005. New sanitation techniques for controlling tephritid fruit flies (Diptera: Tephritidae) in Hawaii. *Journal of Applied Science and Environmental Management* 9, 5–14.
- Landis, D.A., Wratten, S.D., Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45, 175–201.
- Le Pelley, R.H., 1973. Coffee insects. *Annual Review of Entomology* 18, 121–142.
- Maldonado, C.E., 2007. Variabilidad genética y evaluación biológica del parasitoid *Prorops nasuta* Waterston en Colombia. University of Pamplona, Colombia. M.Sc., thesis, p. 71.
- Murphy, S.T., Day R., Orozco, J., Kucel P., 2001. Investigations into the use of *Heterospilus coffeicola* for the biological control of the coffee berry borer in Latin America and Africa. Unpublished report for the period Nov 2000–July 2001, CABI Commodities, Ascot, UK, p. 13.
- OILB, 1971. Liste d'identification des entomophages 8. OILB, Genève.
- Pérez-Lachaud, G., Hardy, I.C.W., Lachaud, J.P., 2002. Insect gladiators: competitive interactions between three species of bethylid wasps attacking the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Biological Control* 25, 231–238.
- Pérez-Lachaud, G., Batchelor, T.P., Hardy, I.C.W., 2004. Wasp eat wasp: facultative hyperparasitism and intra-guild predation by bethylid wasps. *Biological Control* 30, 149–155.
- Pérez, J., Infante, F., Vega, F.E., 2005. Does the coffee berry borer (Coleoptera: Scolytidae) have mutualistic fungi? *Annals of the Entomological Society of America* 98, 483–490.
- Perfecto, I., Mas, A., Dietsch, T., Vandermeer, J., 2003. Conservation of biodiversity in coffee agroecosystems: a tri-taxa comparison in Southern Mexico. *Biodiversity and Conservation* 12, 1239–1252.
- Purcell, M.F., Jackson, C.G., Long, J.P., Batchelor, M.A., 1994. Influence of guava ripening on parasitism of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), by *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) and other parasitoids. *Biological Control* 4, 396–403.
- Quintero, C., Bustillo, A.E., Benavides, M.P., Chaves, C.B., 1998. Evidencias del establecimiento de *Cephalonomia stephanoderis* y *Prorops nasuta* (Hymenoptera:

- Bethylidae) en cafetales del departamento de Nariño, Colombia. *Revista Colombiana de Entomología* 24, 141–147.
- Richter, A., Klein, A.M., Tschardt, T., Tylianakis, J.M., 2007. Abandonment of coffee agroforests increases insect abundance and diversity. *Agroforestry Systems* 69, 175–182.
- Salazar, M.R., Chaves, B., Riaño, N., Arcila, J., Jaramillo, R.A., 1993. Crecimiento y desarrollo del fruto del café *Coffea arabica* L. var. Colombia. *Revista Cenicafé* 45, 41–50.
- SAS Institute Inc., 1999. SAS/STAT User's Guide. SAS Institute Inc., Cary, North Carolina.
- Shannon, C.E., 1948. A mathematical theory of communication. *The Bell System Technical Journal* 27, 379–423 and 623–656.
- Ticheler, J., 1961. Etude analytique de l'épidémiologie du scolyte des graines de café *Stephanoderis hampei* Ferr. en Côte d'Ivoire. *Mededelingen Landbouwhogeschool Wageningen* 61, 1–49.
- van den Bosch, R., Telford, A.D., 1964. Environmental modification and biological control. In: DeBach, P. (Ed.), *Biological Control of Insect Pests and Weeds*. Chapman and Hall, London, UK, pp. 459–487.
- van Driesche, R.G., 1983. Meaning of "percent parasitism" in studies of insect parasitoids. *Environmental Entomology* 12, 1611–1622.
- van Emden, H.F., Service, M.W., 2004. *Pest Vector Control*. Cambridge University Press, UK.
- Vega, F.E., 2004. Coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). In: Capinera, J.L. (Ed.), *Encyclopedia of Entomology*, vol. 1. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 575–576.
- Vega, F.E., Mercadier, G., Damon, A., Kirk, A., 1999. Natural enemies of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) in Togo and Côte d'Ivoire, and other insects associated with coffee beans. *African Entomology* 7, 243–248.