Biology, Ecology and Biological Control of the Coffee Berry Borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae)

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Contents

Abbreviations.....i

Summary.....iii

Zusammenfassung.....vi

General introduction.....1

1. **Coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae): Searching for sustainable control strategies……..6**

Introduction……..……..7

Basic biology of *H. hampei*……....9

Biological control of *H. hampei* ……..13

Implication for *H. hampei* IPM……...……26

Some issues surrounding coffee, *H. hampei*, IPM and sustainability……..29

2. **Biological control of the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae) by *Phymastichus coffea* LaSalle (Hymenoptera: Eulophidae) in Colombia……32**

Introduction……..……33

Material and Methods……...35

Results……..39

Discussion……45

3. **Field superparasitism by *Phymastichus coffea*, a parasitoid of adult coffee berry borer, *Hypothenemus hampei*……48**

Introduction……..49

Material and Methods……...……51

Results……..55

Discussion……60

4. **Parasitoids of the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae) in Kenya: a two-year exploration…….65**

Introduction……..66

Material and Methods……...68

Results……..……72

Discussion……81

5. ***Aphanogmus* sp (Hymenoptera: Ceraphronidae) hyperparasitoid of *Prorops nasuta* Waterston (Hymenoptera: Bethylidae) parasitoid of the coffee berry borer in Kenya……86**

6. **Molecular elucidation of the role of predatory thrips for biological control of the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae)…….91**

Introduction..……92

Material and Methods……..94

Results and discussion…….99

7. **Development of a new laboratory production technique for Coffee Berry Borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae), using fresh coffee berries…….....107**

Introduction……108

Material and Methods…….110

Results……114

Discussion……..……120

8. **Thermal tolerance of the coffee berry borer *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae: Scolytinae): inferences of climate change impact on a tropical insect pest……..124**

Introduction……125

Material and Methods……127

Results……133

Discussion……..150

General discussion.....158

Appendix.....177

References.....179

Acknowledgments

Curriculum Vitae

Declaration by candidate

Abbreviations

AFLP	Amplified Length Polymorphism
ANOVA	Analysis of Variance
Approx.	Approximately
Bp	Base Pair
CBB	Coffee Berry Borer
CENICAFE	Centro Nacional de Investigaciones de Café
COI	Cytochrome Oxidase I
CRF	Coffee Research Foundation
d.f	Degrees of Freedom
DAF	Days After Flowering
DD	Degrees-days
EPF	Entomopathogenic Fungi
EPN	Entomopathogenic Nematodes
F1	First Generation
GLM	General Linear Model
ICIPE	International Centre of Insect Physiology and Ecology
ICO	International Coffee Organization
IPCC	Intergovernmental Panel on Climate Change
IPM	Integrated Pest Management
KARI	Kenya Agricultural Research Institute
L: D	Light Dark Period
L1	First Instar Larvae
L2	Second Instar Larvae
LSM	Least Square Means

m.a.s.l.	Meters Above the Sea Level
Max.	Miximum
Min.	Minimum
Mm	Milimiter
μL	Microliter
μM	Micromole
μm	Micrometer
NS	Non Significant
PCR	Polymerase Chain Reaction
Pers. Comm.	Personal Communication
Re-Re	Recoleccion and Repase
RH	Relative Humidity
SEL	Systematic Entomology Laboratory
TSM	Thermal Safety Margin
USDA	United States Department of Agriculture
Var.	Variety
WT	Warming Tolerance
χ^2	Chi-Square

Summary

Coffee (mainly *Coffea arabica* and *C. canephora*), the world's most valuable tropical export crop, on which more than 100 million people in the tropics depend for their livelihood, is severely affected by its main pest the coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari). The studies presented in this thesis aim at contributing to an improved insight into the biology and ecology of CBB, and indicate new avenues for integrated and biological control of the pest. Chapter 1 summarizes the present knowledge on CBB in a comprehensive literature review. The potential of the eulophid parasitoid *Phymastichus coffea* LaSalle to control *H. hampei* populations under field conditions in Colombia is presented in chapters 2 and 3. Parasitism and superparasitism of CBB by *P. coffea* is significantly affected by the age of the berries at the time of CBB infestations, and by the position of CBB inside the berries. Increasing the time of *P. coffea* releases after the artificial CBB infestations led to decreased levels of parasitism/superparasitism in CBB. Under fields conditions, 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 parasitism and superparasitism of *H. hampei* by *P. coffea* and thus efficacy of this natural enemy to control CBB in the field. Chapters 4, 5 and 6 report on an extensive search for new natural enemies of *H. hampei* in Kenya. After two-year field study in the western part of the country the bethylid *Prorops nasuta* Waterston proved to be the most important, effective, and dominant parasitoid of *H. hampei*, with CBB-infested coffee berries that have fallen to the ground being the main source of its natural enemies. Consequently we hypothesize that the hugely successful cultural control practice of crop sanitation in the Americas, which is the backbone of CBB IPM, may be largely affecting the performance of *P. nasuta* in countries where the parasitoid has been released. In addition, this two-year search for natural enemies yielded two new records of insects associated with *H. hampei*. The first is *Aphanogmus* sp., a hyperparasitod of *P. nasuta*. It is a gregarious ectoparasitoid of larval and pupal stages of *P. nasuta*, with a distinct emergence pattern that follows its host. Under field conditions in Western Kenya around 10% of *P. nasuta* immature

stages were found to be parasitized by *Aphanogmus* sp. The second discovery is a new natural enemy of *H. hampei*, and most likely the first ever recorded predator of CBB in Africa. *Karnyothrips flavipes* Jones (Thysanoptera: Phaelothripidae) was observed preying upon immature stages of *H. hampei* inside the infested berries collected from the ground. Field observations, laboratory trials and molecular tools have confirmed the role of *K. flavipes*, as a predator of CBB in Western Kenya. The females oviposit up to 29 eggs inside an individual coffee berry, and after hatching, larvae and adults spend most of their life-time inside the CBB galleries preying on *H. hampei*. The potential of *K. flavipes* as a biological control agent of *H. hampei* is discussed. In chapter 7 the suitability of a mixture of plaster of Paris and charcoal as a means to regulate the moisture content of coffee berries and the relative humidity (moisture conditions) of the rearing environment and its impact on rearing CBB was evaluated under laboratory conditions. Significantly higher survival and progeny production was achieved when using this methodology compared to the vials that did not contain the plaster of Paris mixture regardless of the quality of the coffee used, as shown by the 6-7-fold increase in survivorship of the F1 and an average of 100 individuals per berry vis-à-vis 1.7 in the control. This rearing methodology is specially suited to conduct experiments on the biology and behaviour of CBB under controlled conditions in the laboratory.

The development of the aforementioned methodological set-up made possible to determine the, until now, unknown thermal tolerance of *H. hampei* and enabled us to make inferences on the possible effects of climate change on the insect using climatic data from Colombia, Kenya, Tanzania, and Ethiopia. The extremes for *H. hampei* survival are 15 and 30°C, but development takes place only between 20 and 30°C. Our thermal tolerance estimates indicate that one strong reason why the insect is not present in certain regions of Ethiopia is the low mean annual minimum temperatures prevalent there, and not plant resistance, natural enemies, etc, as previously speculated. Our model suggest that a small increase in temperature will lead to faster insect development and based on the fact that *H. hampei* feeds solely on coffee, it will likely track any latitudinal and/or altitudinal movement of the crop, leading to increased pest pressure and yield losses in the reduced coffee production areas of the world. However, the negative effects of climate change on coffee

production could be alleviated by increased usage of shade trees in coffee plantations. We therefore conclude that in the future coffee should be grown as it originally evolved in the forests of Africa, i.e., as an understory plant. A proactive strategy to cope with climate change will lead to lower losses by *H. hampei* due to cooler plantations, and will have the added benefits of lower deforestation, and increased biodiversity.

Key words: *Coffea arabica, C. canephora*, coffee berry borer, *Hypothenemus hampei*, rearing, total progeny, biological control, parasitoid, superparasitism, hyperparasitoid, cultural control, IPM, predator, predatory thrips, sustainable production bionomics, temperature, climate change, shade grown coffee.

Zusammenfassung

Kaffee, vornehmlich *Coffea arabica* L. und *C. canephora* L. (Rubiaceae), ist weltweit das ökonomisch bedeutsamste tropische Exportprodukt. Mehr als 100 Millionen Menschen in den Tropen hängen in ihrer Existenz direkt oder indirekt von der Kaffeeproduktion ab. Der Kaffeekirschenbohrer *Hypothenemus hampei* (Ferrari) ist global der wichtigste Kaffeeschädling, und die Untersuchungen in dieser Dissertation versuchen einen Beitrag zum besseren Verständnis der Biologie und Ökologie des Käfers zu leisten, sowie neue Möglichkeiten zu seiner integrierten und biologischen Bekämpfung aufzuzeigen.

Kapitel 1 beinhaltet den jüngsten Literatur-review des Schädlings. Kapitel 2 und 3 beschäftigen sich mit dem Potential der Eulophide *Phymastichus coffea* LaSalle, einem Parasitoiden von *H. hampei*, unter Feldbedingungen in Kolumbien. Parasitismus und Superparasitismus von *H. hampei* durch *P. coffea* werden signifikant von dem Alter der Kaffeekirschen zum Zeitpunkt des Befalls durch den Käfer, sowie von der Position von *H. hampei* in der Kaffeekirsche beeinflusst. Je länger der zeitliche Abstand zwischen den *P. coffea* Freilassungen und der künstlichen Infestation von *H. hampei* war, umso niedriger das Ausmaß von Parasitismus und Superparasitismus des Käfers. Unter Feldbedingungen bestimmen entwicklungsbedingte Effekte der Kaffeekirschen, die wiederum das Verhältnis zur Verfügung stehender Wirte für die Parasitoide determinieren, den Einfluss von Parasitismus und Superparasitismus und somit die Effizienz der Wespen als natürliche Feinde von *H. hampei*.

Kapitel 4, 5 und 6 beschreiben umfangreiche Explorationen zu bekannten und neuen Antagonisten von *H. hampei* in Kenia. In zweijährigen Freilanduntersuchungen im westlichen Teil des Landes entpuppte sich die Bethylide *Prorops nasuta* Waterston als mit Abstand der wichtigste und effizienteste Parasitoid von *H. hampei*. Gefallene, reife Kaffeekirschen in der Streuschicht waren das wichtigste Reservoir für den Parasitoid. Auf Grund dessen ist es möglich dass in Lateinamerika weit verbreitete Hygienemaßnahmen in Kaffeeplantagen wie das Entfernen von

abgefallenen Kaffeekirschen zur *H. hampei* Bekämpfung, das Kontrollpotential von *P. nasuta* stark beeinträchtigen.

Des weiteren konnten in dieser Freilandstudie zwei neue mit *H. hampei* assoziierte Insekten identifiziert werden. Bei dem ersten handelt es sich um die Ceraphronide *Aphanogmus* sp., einem Hyperparasitoden von *P. nasuta*. Es ist ein gregärer Ectoparasitoid von *P. nasuta* Larven und Puppen dessen Phenologie zeitversetzt der seines Wirtes gleicht. Unter Feldbedingungen im Westen Kenias erwiesen sich durchschnittlich 10% der Juvenilstadien von *P. nasuta* als von *Aphanogmus* sp. parasitiert. Bei dem zweiten Insekt handelt es sich um einen neuen natürlichen Feind von *H. hampei* und möglicherweise um den ersten Nachweis eines spezialisierten Prädators des Schädlings. Larven und Adulte von *Karnyothrips flavipes* Jones (Thysanoptera: Phaelothripidae) ernähren sich in den Kaffeekirschen von Eiern, Larven und Puppen von *H. hampei*. Verhaltensstudien im Feld und Labor sowie molekulare Untersuchungen bestätigten dass es sich bei *K. flavipes* um einen Prädator des Kaffeekirschenbohrers handelt. Die weiblichen Thripse legen bis zu 29 Eier pro Kaffeekirsche, und nach dem Schlupf verbringen Adulte und Larven die meiste Zeit in den *H. hampei* Galerien auf der Jagd nach Beute. Die potentielle Bedeutung von *K. flavipes* als natürlicher Feind von *H. hampei* wird diskutiert.

Kapitel 7 beschreibt die Entwicklung einer neuen Methode zur Laborzucht von *H. hampei* auf frischen Kaffeekirschen, dem natürlichen Substrat des Käfers. Hierfür wurde eine Mischung aus Gips und Aktivkohle verwandt, um den Feuchtegehalt der Kaffeekirschen und die relative Luftfeuchtigkeit der Zuchtcontainer besser zu steuern. Unabhängig von der Qualität des Kaffees wurden signifikant höhere Überlebensraten (6- bis 7-fach) und Nachkommenschaften (100 Nachkommen pro Kaffeekirsche vis-à-vis 1.7 in der Kontrolle) mit der neuen Methode im Vergleich zur Kontrolle nachgewiesen. Diese Methode ist besonders gut für detaillierte Laborstudien zur Biologie und zum Verhalten von *H. hampei* unter kontrollierten Bedingungen geeignet.

Die Entwicklung dieser Methodik ermöglichte es die bis dato unbekannten Temperaturschwellenwerte (thermal tolerance) von *H. hampei* zu bestimmen, und Rückschlüsse auf den möglichen Einfluss von Klimawandeleffekten auf den Schädling zu ziehen (Kapitel 8). Hierfür wurden langjährige meteorologische

Datensätze aus Kolumbien, Kenia, Tansania und Äthiopien verwandt. Die kritischen Temperaturschwellenwerte für das Überleben von *H. hampei* sind 15 und 30°C, aber der Käfer entwickelt sich nur zwischen 20 und 30°C. Diese Temperaturschwellenwerte erklären warum *H. hampei* in bestimmten Regionen Äthiopiens nicht vorkommt, da dort die durchschnittlichen jährlichen Minimumtemperaturen zu niedrig sind für das Insekt. Das hier entwickelte Modell lässt vermuten dass schon ein kleiner Temperaturanstieg zu einer beschleunigten Entwicklung von *H. hampei* führen wird. Auf Grund der Tatsache dass *H. hampei* ausschließlich Kaffee befällt, kann man davon ausgehen dass der Schädling seiner Wirtspflanze folgen wird falls diese als Folge eines Klimawandels in nörd- oder südlicheren Breitengraden oder in größeren Höhenlagen angebaut wird, was konsequenterweise zu höheren Verlusten in den weltweit schrumpfenden Kaffeeanbaugebieten führen könnte. Diese negativen Auswirkungen eines Klimawandels könnten allerdings deutlich reduziert werden, wenn Kaffee wieder vermehrt in Plantagen unter Schattenbäumen angebaut würde, entsprechend seiner Natur als Unterholzbaum aus den Wäldern Afrikas. Eine solche vorausschauende Strategie würde zu verringerten Verlusten durch *H. hampei* auf Grund von niedrigeren Temperaturen in den Plantagen führen, und zugleich einen Beitrag zur Reduktion von Entwaldung und zur Erhöhung der Biodiversität in Agrarökosystemen leisten.

Schlagwörter: *Coffea arabica, C. canephora*, Kaffeekirschen, Kaffeekirschenbohrer, *Hypothenemus hampei*, biologische Bekämpfung, Parasitoid, *Phymastichus coffea, Prorops nasuta*, Superparasitismus, Hyperparasitoid, *Aphanogmus* sp., integrierte Bekämpfung, Prädator, räuberischer Thrips, *Karnyothrips flavipes*, Zuchtverfahren, Feuchtegehalt, Klimawandel, Temperaturschwellenwerte, Schattenbäume.

General Introduction

Coffea spp is one of the predominant genera in the family Rubiaceae that includes more than 400 species (Davis et al., 2006). Economically the most important *Coffea* species are *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* Pierre ex A. Froehner (Robusta coffee) (Berthaud and Charrier, 1988). Arabica and Robusta coffee are worldwide the economically most important agricultural commodities (Clifford and Wilson, 1985), with an annual retail value exceeding US $ 70 billion (Vega, 2008), only surpassed by petroleum products. In the tropics, coffee is produced in more than 80 countries on an estimated area of 10 million hectares where more than 100 million people, most of them small-scale farmers earning less than 2 $ a day, depend on it for their livelihoods (Vega, 2008).

Arabica coffee, considered the highest quality coffee, is presumed to be native to the forests of South Western Ethiopia where it naturally grows as an understory tree between 1,600 and 2,800 meters above sea level (m.a.s.l) (Wellman, 1961; Davis et al., 2006). Robusta coffee on the other hand, is native to lowland forests of the Congo River Basin, where it similarly grows as an understory tree at altitudes ranging from 0-1200 m.a.s.l. (Davis et al., 2006).

Endemic to Africa, the coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) is the most devastating insect pest of commercial coffee (Le Pelley, 1968; Damon, 2000; Jaramillo et al., 2006). The insect causes serious economic losses affecting worldwide more than 20 million rural households (Vega et al., 2003). Female *H. hampei* bore galleries into the endosperm of the coffee berries where they oviposit more than 100 eggs, resulting in both

qualitative and quantitative losses through larval feeding and oviposition gallery construction by the females (Le Pelley, 1968; Decazy, 1990; Damon, 2000). This cryptic life history of CBB inside the coffee berry, combined with a skewed sex ratio favouring females (10:1) and sibling mating inside the berry (Brun et al., 1995) makes the pest extremely difficult to control. Reported infestation levels of *H. hampei* can range from 50-90%, e.g., 60% in Colombia, 58-85% in Jamaica, 50-90% in Malaysia, and 60% in Mexico (Vega, 2004). In **Chapter 1** of this thesis a detailed literature review on the ecology, biology and control of *H. hampei* is presented.

To date CBB is present in all coffee growing regions of the world, except for Hawaii (Vega, 2004). With regard to the Americas, one of the most productive coffee growing regions in the world (ICO, 2008), the pest was first accidentally introduced to Brazil in 1913 (Bergamin, 1943). Because of the specific climatic conditions in the coffee growing areas of Brazil, the high prevalence of mechanical harvest and the dry processing of the coffee there, CBB never reached a significant pest status there. Several decades after this introduction *H. hampei* started to spread to other coffee producing countries in Latin America like Ecuador, Bolivia, Colombia, Mexico, Guatemala, Honduras, and El Salvador (Bustillo, 2002). Contrary to Brazil in these countries CBB immediately became the main threat to coffee production.

As a response coffee growers in the Americas tried to combat CBB predominantly through use of broad-spectrum insecticides like endosulfan and chlorpyrifos. However, these insecticides are highly toxic and a threat to the environment, the farmers who use them, and the communities living adjacent to treated coffee plantations (Baker et al., 2002). Moreover, because of the concealed nature of *H. hampei* chemical control in general is not very effective. As an exotic

outbreak pest in the Americas, classical biological control was considered a promising avenue. Consequently, searches for natural enemies in West and East Africa were conducted (Baker, 1999). During the last one hundred years the following natural enemies, all of them parasitoids, of *H. hampei* were discovered during explorations in Africa: the braconid *Heterospilus coffeicola* Schmiedeknecht and the bethylid *Prorops nasuta* Waterston by Hargreaves (1926) and Hempel (1934) in Uganda; the bethylid *Cephalonomia stephanoderis* Betrem by Ticheler (1961) in Ivory Coast; and the eulophid *Phymastichus coffea* LaSalle by Borbón-Martinez (1989) in Togo and described by LaSalle (1990). Except for *H. coffeicola* for which to date no viable rearing protocol has been developed, all the other parasitoids have been introduced to the Americas (Barrera et al., 1990; Baker, 1999; Dufour et al., 1999).

Probably the best-documented case of a CBB classical biological control program in the Americas comes from Colombia (Baker, 1999). There CBB was first recorded in August 1988 (Bustillo et al., 1998), and to date is widespread throughout all coffee growing regions of the country and is considered to be the number one pest (Bustillo et al., 1998). The two larval-pupal ectoparasitoids *C. stephanoderis* and *P. nasuta* were introduced into Colombia in the late 1980ies (Baker, 1999). They were first tested under laboratory conditions and subsequently released in the field (Benavides et al., 1994; Portilla and Bustillo, 1995). Later in 1996 the eulophid *P. coffea*, was introduced to Colombia and its establishment was reported in 1998 (Baker, 1999; Aristizabal et al., 2004). *P. coffea* is a gregarious endoparasitoid of CBB females (Borbón, 1989). The parasitoid usually attacks the female beetles before the damage to the coffee endosperm has taken place. Among others, this trait of the parasitoid made it a very promising candidate for biological control of CBB

(Gutierrez et al., 1998). **Chapters 2 and 3** of this thesis report on some of the research carried out in Colombia to elucidate the potential of *P. coffea* as a CBB biological control agent.

In spite of all the efforts to control the pest in the countries where it has accidentally been introduced through the development of integrated pest management (IPM) programs involving, among others, augmentative releases of the introduced parasitoids, applications of entomopathogenic fungi like *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), trapping, and cultural control, CBB is still the main biotic constrain for coffee production in most of the affected countries, with farmers continue to rely mainly on chemical control strategies.

For instance, although, *C. stephanoderis, P. nasuta* and *P. coffea* became established in the release countries (e.g., Mexico, Colombia and India), their impact on *H. hampei* field populations has been limited to 5% or less (Quintero et al., 1998; Baker, 1999; Infante et al., 2001). On the other hand, the rather effective cultural control strategy for CBB in Colombia is labour intensive and thus costly (Duque and Baker, 2002). There are also growing environmental concerns on the use of endosulfan and chlorpyrifos for CBB control, and increasing problems with insecticide resistance (Gongora et al., 2001). Moreover, the sustainable coffee production and certification schemes stress the safety aspects of pest control, resulting in an increased demand for biological control solutions and new research for environmentally more friendly control strategies against CBB (Jaramillo et al., 2006). Consequently a large part of the here reported investigations (**Chapters 4, 5 and 6**) were devoted to an intensive search for new natural enemies of *H. hampei* in Kenya.

One of the prerequisites for successful biological control of a pest in an introduced area is a sound understanding of its general biology and ecology (van Driesche and Bellows, 1996). In spite of the economic importance of the pest, there are still major gaps in our understanding of the biology and ecology of *H. hampei*. For instance conflicting data on the bionomics of the pest are reported in the literature (i.e., Bergamin, 1943; Ticheler, 1963; Decazy, 1990; Barrera 1994; Montoya and Cardenas, 1994; Ruiz, 1996; Fernandez and Cordero, 2007). These differences are most likely due to the difficulties of studying a concealed pest like CBB under controlled conditions and suggest problems with existing methodologies (Damon, 2000). Because of the absence of a viable protocol to maintain *H. hampei* on its natural substrate, fresh coffee berries in the laboratory, some of the previous studies were conducted under field conditions. Yet varying environmental factors lead to variations in recorded biological parameters of the insect (e.g. Ruiz, 1996; Fernandez and Cordero, 2007). In **Chapters 7** we are describing a new laboratory methodology for CBB rearing on fresh coffee berries. This allowed us finally to conduct an extensive study on the thermal tolerance of *H. hampei* and to make inferences on the potential impact of global warming on the pest using climatic data from coffee growing areas in Colombia and three East African countries (**Chapter 8**).

CHAPTER 1

Coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae): Searching for sustainable control strategies*

Abstract

The coffee berry borer *Hypothenemus hampei* (Ferrari) is the most serious pest of the world's most valuable tropical export crop. Since the last review on this insect was published six years ago, many new studies have contributed to an improved insight into the biology and ecology of the beetle, and have indicated new avenues for integrated and biological control. The latest developments in research, both laboratory and field, on the pest, its natural enemies and their implications for integrated control of *H. hampei* are summarized, with a particular focus on the situation in The Americas. Lately, the global coffee industry has changed radically; it has suffered a long cycle of lowest-ever world market prices caused by overproduction and technological change. At the same time, the advent of sustainable certification schemes has had a major impact on the industry. The role of integrated pest management and biological control of *H. hampei* in an era of changes in the coffee industry is discussed.

Keywords: Coffee; Biological Control; IPM; *Hypothenemus hampei*; Parasitoids.

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Introduction

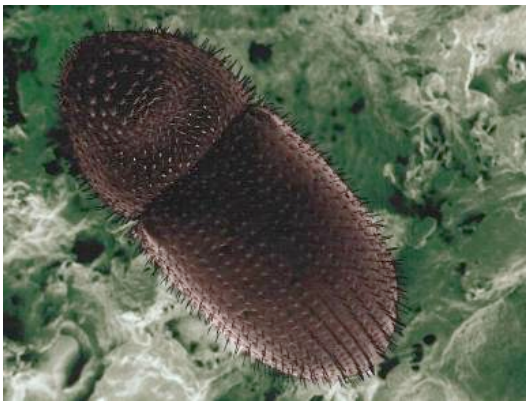
In 70 countries in the humid tropics, coffee (*Coffea* spp., Rubiaceae) is the most important agricultural commodity. Its production has increased over the last decades through the use of high yielding varieties, fertilisers and high density planting (Baker et al., 2002). However, in many countries coffee production is severely threatened by a number of pests and diseases. The most important of the insect pests is the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), (Le Pelley, 1968; Damon, 2000). *H. hampei* causes serious economic losses and affects the economy of more than 20 million rural families in the world (Fig. 1) (Vega et al., 2003a).

Figure 1. Countries with known record of *Hypothenemus hampei*. Note: dots do not indicate the precise location where the pest was initially recorded or its present area of distribution within the respective countries.

Extremely high levels of infestation in untreated plantations have been reported, e.g. Uganda 80%, Colombia 60%, Jamaica 58-85%, Tanzania 90%, Malaysia 50-90% and Mexico 60% (Vega, 2004). Presently many coffee farmers rely on the application of synthetic insecticides for control of *H. hampei*. Yet, endosulfan and chlorpyrifos, the two most commonly used insecticides against *H. hampei*, are highly toxic and a threat to the environment, the farmers who use them, and the communities living adjacent to treated coffee plantations (Baker et al., 2002). Growing environmental concerns and increasing problems with insecticide resistance in *H. hampei* (Brun et al., 1989; Gongora et al., 2001) have stimulated the search for environmentally more friendly control strategies against the pest**.** Since the most recent review on *H. hampei* (Damon, 2000) many new reports have contributed to a better understanding of the biology and ecology of *H. hampei*, as well as indicating new avenues for biological pest control. During this same period, the coffee industry has changed radically; it has suffered a long cycle of lowest-ever world market prices caused by overproduction and technological change (Varangis et al., 2003). At the same time the advent of sustainable certification schemes (Giovannucci and Koekok, 2003) has also had a major impact on the coffee industry. In this review we will focus on latest discoveries on the biology and genetics of the beetle, and give a special emphasis on new findings on biological control of *H. hampei* primarily in The Americas. Finally, the role of IPM and biological control of *H. hampei* in an era of changes in the coffee industry is discussed.

Basic biology of *H. hampei*

The basic biology and ecology of *H. hampei* has been extensively reviewed by Damon (2000). Females (1.4 - 1.6 mm long) attack developing coffee berries from about eight weeks after flowering up to harvest time (> 32 weeks) (Baker, 1999). They bore galleries into the endosperm of the coffee berries (Fig. 2), causing two types of damage, i.e. premature fall of young berries, and qualitative and quantitative losses in coffee through feeding of the gregarious larvae inside the berries (Le Pelley, 1968) (Figure 2).


a


b

Figure 2. a) Female of the coffee berry borer *Hypothenemus hampei*; b) female CBB penetrating a coffee berry; c) larvae, pupae and males CBB inside a coffee berry. (Photos: a) Fernando E. Vega USDA; b) Gonzalo Hoyos CENICAFE).

Each berry is attacked by a single female (often referred to as the 'colonising female'), and oviposition inside the galleries takes place over a period of 20 days; the female daily lays two to three eggs inside the berry (Bergamin, 1943). The population dynamics of, and the infestation pattern by *H. hampei* are closely related to climatic factors like precipitation and relative humidity (Baker et al., 1992), as well as to the physiology of the coffee plant (Salazar et al., 1993; Ruiz, 1996). The dry matter

content of the endosperm is the most crucial factor determining the attack by *H. hampei* and its speed of penetration into the coffee berry. Seeds with < 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 the sufficient amount of dry matter content for the development of her offspring (Alonzo, 1984). The female stays with her brood and does not leave the berry (Baker et al., 1992). Females of the first brood either leave the berry after having mated with their male siblings inside the berries (Bustillo et al., 1998), or after mating stay permanently in the berry and egg-laying resumes (Baker et al., 1992). The males do not abandon the berry (Ticheler, 1961). The emergence of the searching females from the berries is triggered by high temperature and relative humidity (Baker et al., 1992).

Recent advances in *H. hampei* biology

Wolbachia in *H. hampei*

Wolbachia are cytoplasmatically inherited proteobacteria found in the reproductive tissue of a wide range of arthropods, i.e. insects, isopods and mites; they can cause sex ratio distortions (Werren, 1997). *Wolbachia* cause the induction of parthenogenesis, cytoplasmatic incompatibility, male-killing and the conversion of male individuals into functional females (Stouthamer et al., 2002). In 2002 a species of *Wolbachia* was detected in *H. hampei* populations from Brazil, Colombia, Ecuador, India, Nicaragua, El Salvador, Benin, Honduras, Mexico and Uganda (Vega et al., 2002). These investigations suggest that the presence of *Wolbachia* might be one reason behind previous findings on the female-biased sex ratio of *H. hampei* (≈ 10:1)

and on its functional haplodiploidy, i.e. the fact that both sexes are diploid but that only males transmit their maternally derived chromosomes to the offspring (Brun et al., 1995). However, in spite of its skewed sex ratio, no *Wolbachia* was detected in *H. hampei* populations from East Africa, the probable centre of origin of the pest (Vega et al., 2002). Though haplodiploid organisms often have female-biased sex ratios without any involvement of *Wolbachia*; this includes pseudoarrhenotokous species such as predatory mites that probably have similar genetic mechanisms of sex determination to *H. hampei* like paternal genome loss (Sabelis et al., 2002).

Biogeography of the coffee berry borer

Benavides et al. (2005), using amplified fragment length polymorphism (AFLP) DNA fingerprints, studied the diversity and biogeography of *H. hampei* and revealed low levels of genetic variability in beetles of different geographic origins, confirming previous findings by Andreev et al. (1998). These low levels of genetic variability of *H. hampei* have important pest control implications as they suggest that resistance to chemicals, if it were to emerge, would presumably become widespread much faster due to high levels of inbreeding (Brun and Suckling, 1992; Brun et al., 1995). Similar to Bergamin (1944), Benavides et al. (2005) also hypothesised that all accidental introductions of *H. hampei* into The Americas derived from West African source populations. They found the greatest match between fingerprints from South America and Africa in samples taken in Cameroon. However, the authors only sampled in two geographically rather adjacent locations in Cameroon, whereas for example in Uganda samples were taken in eleven different sites. Moreover, the authors strangely

attributed Cameroon to be part of West and not Central Africa, and in previous reports *H. hampei* was believed to originate from Central and Eastern Africa (see review by Ticheler, 1961).

Fungal associations in H. hampei

A mutualistic interaction between some members of the weevil subfamilies Scolytinae and Platypodinae (i.e., bark and ambrosia beetles) (Farrell et al., 2001) and asexual fungi has been extensively studied. In such interaction, the fungi may contribute to the death of the host tree, or the beetles may benefit from the association by feeding on the fungi (Paine et al., 1997). A possible interaction between *H. hampei* and fungi has been hypothesized for many years. Waterson and Norris (1989) speculated that when *H. hampei* first-instar larvae feed on frass produced by their mothers they might acquire a symbiotic fungus. Subsequently Rojas et al. (1999) and Morales-Ramos et al. (2000) reported a symbiosis of the beetle with *Fusarium solani* (Martius) (Moniliales: Tuberculariaceae), and the latter authors hypothesised that *H. hampei* obtains ergosterol, a key substance for the reproduction of the beetle, from the fungus. Morales-Ramos et al. (2000) study was the first to report a mutualistic relationship between *H. hampei* and a microbe. More recently Peterson et al. (2003) identified *Penicillium brocae* sp. n. (Deuteromycotina: Hyphomycetes) in *H. hampei* populations from Mexico. In addition, Carrion and Bonet (2004), studying the mycobiota associated with *H. hampei* and its galleries, reported 13 different fungi. However, seven of them were saprophytes and one was the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes), the latter commonly reported infecting *H. hampei* in the field (Baker, 1999). In a similar

study Pérez et al. (2003) could identify 40 fungal and two yeast species from *H. hampei* and its galleries. Also Vega et al. (2003b) found a yeast species, *Pichia burtonii* Boidin, associated with *H. hampei*. They suspected that the yeast is involved in the breakdown of caffeine, but had to reject this hypothesis of a mutualistic relationship after subsequent laboratory studies. Moreover, most recently Pérez et al. (2005) could demonstrate that *F. solani* and the yeast *Candida fermentati* (Saito) have no effect on reproduction and survival of *H. hampei*, thereby ruling out any mutualistic relationship between *H. hampei* and the fungus and the yeast. Hence unlike in other scolytids (Paine et al., 1997), currently there is no evidence to suggest that *H. hampei* has mutualistic associations with fungi or yeasts.

Biological control of *H. hampei*

Parasitoids for *H. hampei* control

Cephalonomia spp. and *Prorops nasuta*

Classical biological control through introductions of the two bethylid wasps of African origin, *Cephalonomia stephanoderis* Betrem and *Prorops nasuta* Waterston (Figure 3) in South and North America in the 1980s and 1990s (Barrera et al., 1990; Baker, 1999), have not proven entirely successful.

a **b** **c**

Figure 3. Adults of a) *P. nasuta* and b) *C. stephanoderis*, c) larvae of *P. nasuta* attacking a coffee berry borer pupa. (Photos: CENICAFE).

After quarantine in England and studies under laboratory conditions (Barrera et al., 1989; Abraham et al., 1990; Murphy and Moore 1990), the parasitoids were released in the field. Although both parasitoids established in North, Central and South American countries, their impact on field populations of *H. hampei* has been rather limited (Damon, 2000; Baker et al., 2002). After these initial introductions, another *Cephalonomia* sp. native to North America was found naturally attacking field populations of *H. hampei* in Chiapas province of southern Mexico (Pérez-Lachaud, 1998). It was later identified as *C. hyalinipennis* Ashmead, and has a very similar biology to *C. stephanoderis* and *P. nasuta*. All three species are larval-pupal ectoparasitoids of *H. hampei* and usually prey on *H. hampei* eggs; *C. stephanoderis* also attacks and feeds on the adult female *H. hampei*, whereas *P. nasuta* does not attack the beetle for feeding but does use their bodies (abdomens) to block the entrance to infested coffee berries (Lauziere et al., 1999; Infante et al., 2005). Moreover, unlike the two other bethylids that lay only one egg per host,

C. hyalinipennis lays one to four or five eggs per host, and more eggs may be laid in other host species (Pérez-Lachaud, 1998; Pérez-Lachaud and Hardy, 2001). Female *C. hyalinipennis* can live for up to 95 days and their mean fecundity is higher than that of *C. stephanoderis* (Pérez-Lachaud and Hardy, 1999). In laboratory studies considerable inter- and intraspecific competition among *C. stephanoderis*, *P. nasuta* and *C. hyalinipennis* was recorded (Pérez-Lachaud et al., 2002; Batchelor et al., 2005; Batchelor et al., 2006). In general the wasps exhibited aggressive brood and host guarding behaviour, with *C. stephanoderis* being the most successful competitor and often killing its opponents (Batchelor et al., 2005). Additionally, when *C. hyalinipennis* was provided with immature stages of *C. stephanoderis* and *P. nasuta* [but not with the mother of the immature brood], the wasp behaved like a hyperparasitoid (Pérez-Lachaud et al., 2002; Pérez-Lachaud et al., 2004). These authors concluded that *C. hyalinipennis* is a facultative hyperparasitoid of *C. stephanoderis* and *P. nasuta*, and results of their laboratory studies indicate that coexistence among the three parasitoid species might be unlikely based only on fighting behaviour recorded in the laboratory (Pérez-Lachaud and Hardy, 1999; Pérez-Lachaud et al., 2002; Batchelor et al., 2005), but might be possible under field conditions (Batchelor et al., 2006). However, even repeated augmentative releases of only one bethylid species yield levels of parasitism below 5% (Baker, 1999), suggesting that under field conditions the effect of inter- and intraspecific competition in reducing the efficacy of the parasitoids would be insignificant. Nevertheless, even such a small impact of these bethylids on *H. hampei*, when integrated over an entire region, implies a positive economic effect, especially at times of low coffee prices when farmers often limit their more costly control measures. Anecdotal evidence

suggests that when coffee plots are abandoned and the berries not harvested, the populations of bethylid parasitoids increase significantly (P.S. Baker, personal observation), which might limit the invasive impact of the pest on surrounding coffee farms.

Phymastichus coffea

A third parasitoid species introduced to The Americas**,** and subsequently also to India**,** for classical biological control of *H. hampei* is the eulophid *Phymastichus coffea* LaSalle (Fig.4). It was first discovered in Togo in 1987 (Borbón-Martinez, 1989) and later described by LaSalle in 1990. The latter author placed it taxonomically in the subfamily Tetrastichinae. Initially Feldhege (1992) believed it to be the only species in the genus *Phymastichus***,** though later LaSalle (1995) described with *P. xyleborii* LaSalle, a parasitoid of the Hawaiian scolytid *Xyleborus perforans* (Wollaston). The distribution of *P. coffea* ranges from West (e.g. Togo, Benin and Ivory Coast), over Central (Cameroon and Uganda) to East Africa (Burundi and Kenya) (Lopez and Moore, 1998). It is a gregarious endoparasitoid of *H. hampei* adult females (Borbón-Martinez, 1989) (Figure 5). Lopez et al. (1997) described *P. coffea* as an idiobiont and oligophagous parasitoid, though, recently Shaw (2004) proposed the term imagobiont for parasitoids of adult insects.

Fig. 4. Female *Phymastichus coffea*. Photo by Dr Georg Goergen, International Institute of Tropical Agriculture, Benin.

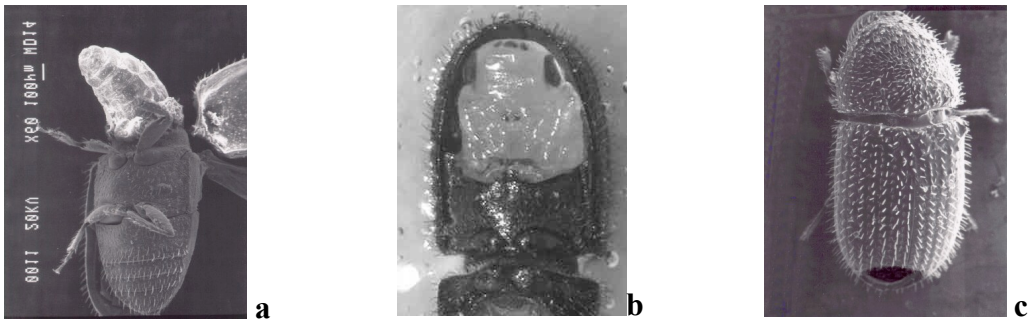


Figure 5. Developmental stages of *Phymastichus coffea* LaSalle. a) larvae; b) pupae; c) mummy of CBB with exit hole. (Photos: a) and c) Dr. H. Arroyave, CIAT; b) P. Baker, CABI-Commodities).

Under laboratory conditions, in addition to *H. hampei*, *P. coffea* parasitizes other *Hypothenemus* spp. like *H. seriatus* (Eichhoff), *H. obscurus* (F.) (Lopez and Moore, 1998), *H. eruditus* Westwood and *H. crudiae* (Panzer) (Castillo et al., 2004a). *P. 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 pre-oviposition period, whereas Feldhege (1992) reported pre-oviposition periods between five minutes and four hours, with 20 minutes as the most frequent duration. *P. coffea* females possess a short and concealed ovipositor (LaSalle, 1990), which obliges them to assume a more or less vertical position on top of the host during oviposition. The oviposition takes between one to seven minutes. A *P. coffea* female can oviposit into the abdomen, thorax or between the thorax and the abdomen of the beetle (Feldhege, 1992) and usually lays two eggs per host, one female and one male (for more detailed information on superparasitism refer to below). A single female offspring develops in the abdomen of the beetle, whereas towards the end of its larval development the male migrates to the head and completes its development there (Lopez et al., 1997). After parasitization, the mobility of the female beetle is greatly impaired; moreover, parasitized females stop ovipositing and usually die after 12 days (Borbón-Martinez, 1989, Feldhege, 1992; Infante et al., 1994). Published data on the duration of the life cycle of *P. coffea* vary to a great extent: Lopez et al. (1997) reported 43 days at 24°C, Feldhege (1992) 30 days at 27°C, and Infante et al. (1994) 27.5 days at 26°C. Under field conditions in Colombia the duration of the life cycle was 46 days at 22°C and 76% relative humidity (Vergara et al., 2001). Likewise, published data on the lifespan of adult males and females vary considerably. Feldhege (1992) reports 30 hours for honey-water fed females and < 22 hours for males at 25 ± 2ºC, whereas Lopez et al. (1997) and Orozco (1997) reported 2-3 and 3-4 days for males and females at 24 ± 1ºC, respectively. In the latter two studies longevity of the females could be extended

to up to 5 days in the laboratory when the parasitoids were fed with a honey-water solution.

P. coffea mass-rearing and releases in the field were first carried out in Colombia. Parasitoids were introduced and subsequently released in 1996 and 1997, respectively, and *P. coffea* establishment was reported in 1998 (Baker, 1999). Further releases followed and according to Aristizabal et al. (2004) the parasitoid has established to date on 41 farms in Colombia. Baker et al. (2002) documented additional successful cases of establishment of *P. coffea* in North, Central and South America. Under field conditions in Colombia, Jaramillo et al. (2005a) observed that parasitism of *H. hampei* by *P. coffea* was significantly affected by the developmental stage of the coffee berries and by the position of the beetles inside the berries at the time of the parasitoid releases (Fig. 6).

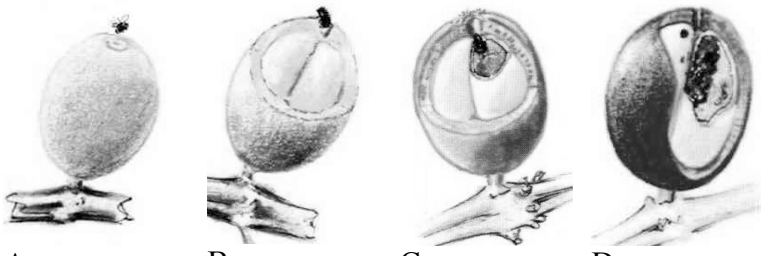


Figure 6. Positions of coffee berry borer *Hypothenemus hampei* in coffee berries (Bustillo *et al.*, 1998; drawing by Gonzalo Hoyos, CENICAFE).

The population dynamics of, and the infestation pattern by *H. hampei* are closely related to climatic factors and the dry matter content of the coffee berries (see section on basic biology of *H. hampei*). Jaramillo et al. (2005a) recorded highest levels of parasitism (85%) in berries younger than 160 days, which equals position B in Fig. 6, thus preventing the *H. hampei* females from reaching the endosperm and

hence damaging the coffee berries. Based on the results of their study Jaramillo et al. (2005a) suggested that the timing of *P. coffea* mass releases in coffee plantations should depend on the age of the berries, to assure that the majority of the beetles have not yet reached the endosperm of the coffee berries at the time of the parasitoid releases. This can be comparatively easy determined through site-specific data on the major blossoming period and also on long-term climatic data since a heavy rain followed by a prolonged dry period usually triggers the blossoming of the coffee tree (de Alvim, 1960). In Colombia, the well-distributed rainfall pattern leads to many flowerings and may present greater difficulties in assessing control points based on the fruiting phenology of the coffee plant.

In laboratory choice experiments Castillo et al. (2004b) observed that *P. coffea* discriminates between parasitized and unparasitized hosts. These authors hypothesised that a marking pheromone is involved in this process and concluded that the ability to discriminate would increase the efficiency of the parasitoid in the field by avoiding superparasitism. However, Jaramillo et al. (2005b) recorded considerable levels of superparasitism by *P. coffea* under field conditions in Colombia. For instance, often more than six *P. coffea* larvae were found in a single host, and these authors could show that the decision of the female to superparasitise is complex and affected by the age of the coffee berries, i.e. its dry matter content, which influences the ratio of available female hosts to searching parasitoids by providing refugees to the herbivore. Thus in summary, though many aspects of the basic biology of the parasitoid are still unknown, *P. coffea* so far appears to be a candidate for biological control of *H. hampei*, especially because it primarily attacks adult female *H. hampei*

outside the berries before they have started ovipositing into the endosperm, i.e. before the coffee beans have been damaged.

Compatibility of P. coffea *with other* H. hampei *control methods*

Within an IPM context, *H. hampei* control methods are divided into two main categories: i) methods targeting *H. hampei* populations when they start to penetrate the coffee berries (positions A and B in Fig. 6) like applications of synthetic or microbial insecticides, and ii) methods that aim at *H. hampei* stages inside the coffee berries like releases of the bethylid parasitoids *C. stephanoderis* and *P. nasuta* (Bustillo et al., 1998). Studies on *P. coffea* so far indicate that the parasitoid prefers to attack *H. hampei* females that are just starting to penetrate the coffee berries. Hence, other *H. hampei* control methods that target females in positions A and B (Fig. 2) will most likely negatively affect *P. coffea*. Studies on releases/ applications of *P. nasuta* and *B. bassiana* and/or synthetic insecticides suggest that the timing of the releases/ applications is of outmost importance and can considerably reduce negative effects on the parasitoids (Mejia et al., 2000; de la Rosa et al. 2000). Comparable studies with *P. coffea* and microbial and/ or synthetic insecticides are needed to evaluate their compatibility and/ or incompatibility as control agents of *H. hampei*.

Mass rearing of P. coffea

P. coffea is presently mass-released in South, Central and North American countries following an augmentative approach that requires high numbers of parasitoid females for field releases. To date the major bottleneck for a high-output rearing of *P. coffea* is the production of large numbers of healthy *H. hampei* females.

So far, two mass-rearing protocols for *P. coffea* have proven successful. The first one was developed by Infante et al. (1994) in México, and the second by the Centro Nacional de Investigaciones de Café (CENICAFE) in Colombia (Orozco, 2002).

Infante et al. (1994) proposed the use of healthy unripe berries for parasitoid rearing. Following their artificial infestation by the beetles, the *H. hampei*-infested berries are then exposed to *P. coffea* females. After approximately 30 days the parasitoids commence to emerge from the mummies.

CENICAFE's mass-rearing technique is slightly more complex and involves two steps, i.e. i) *H. hampei* infestation of premium quality parchment coffee with an initial moisture content of 45%, and ii) their later parasitization by *P. coffea*. During this latter step a gradual reduction in temperature and simultaneous increase in relative humidity is desirable. Using this methodology a complete life cycle of *P. coffea* takes at least 45 days, which is considerably longer than reported by Infante et al. (1994). However, the gradual decrease of temperatures, in addition to a rigorous cleaning of the beans, and the slowly rising relative humidity levels assure that fungal infection and desiccation of the beans are prevented, which would otherwise harm the development of the immature stages of *P. coffea* inside the beetles/ berries. Using this methodology 16 million *P. coffea* adults were produced in CENICAFE's laboratories between 1996 and 2001 (Orozco, 2001).

Though the CENICAFE methodology to mass-produce the wasps has been successful, it is rather costly (Baker, 1999). Hence attempts to mass-produce the hosts using artificial diets have been undertaken. Based on previous work by Villacorta (1985) and Villacorta and Barrera (1993) in Brazil and Mexico, respectively, Portilla (1999a,b) developed in Colombia an easy to produce and significantly cheaper

artificial diet for *H. hampei* called 'Cenibroca'. Presently, using Cenibroca artificial diet hundreds of generations of the beetle hosts and its parasitoids have been mass-produced at low cost at an experimental level, with no significant decline in the fecundity of the insects. Preliminary calculations suggest that augmentative mass releases of *P. coffea* could be economically viable (Baker et al., 2002) if the parasitoid is effective at keeping low populations under control, though this remains to be tested in the field.

Heterospilus coffeicola

Damon (2000) mentioned field observations from Africa that suggest *Heterospilus coffeicola* Schmiedeknecht (Hymenoptera: Braconidae) to be an important natural enemy of *H. hampei*, thus a potentially promising classical biological control agent. Studies by Murphy et al. (2001) in Uganda indicate that *H. coffeicola* females lay only one egg per berry, and that the emerging larva consumes immature stages of *H. hampei*, consequently acting as a predator. However, to date the wasp has not been used in biological control programs against *H. hampei*, which is due to, among others, the so far insurmountable difficulties in rearing the wasps under laboratory or even field conditions (Murphy et al., 2001).

Predators for H. hampei *control*

So far the only known predators of *H. hampei* are ants (Hymenoptera: Formicidae). According to Vega et al. (1999) in Africa *Leptophloeus* sp. near *punctatus* could be a specific predator of *H. hampei*. However, no further studies have been conducted to confirm such specificity. In Colombia Armbrecht et al. (2005) studied ant diversity in shaded coffee plantations where one or several different shade

trees were used and compared it to non-shaded coffee. They found that the number of ant species and their ecological associations and complexity decreased with intensification of coffee production, i.e. less diverse shaded- and non-shaded coffee**.** In non-shaded coffee plantations *Solenopsis picea* Emery and *Pheidole radoszkowski* Mayr could out-compete and exclude other ant species and were found in great numbers and widely distributed in such a production system. In Colombia, seven ant genera have been observed attacking *H. hampei*, i.e. *Solenopsis*, *Pheidole*, *Wasmannia*, *Paratrechina*, *Crematogaster*, and *Brachymyrmex*, with *S. picea* being often the most efficient predator of *H. hampei* in coffee plantations (Bustillo et al., 2002; Armbrecht et al. 2005). They penetrate the infested coffee berries, take out the immature stages of the beetles and transport them to their nests (Bustillo et al., 2002). Additionally, in Mexico Infante et al. (2003) observed that several ant species, for instance *Pseudomyrmex*, *Azteca* and *Tapinoma* spp. (all Hymenoptera: Formicidae), also prey on *P. nasuta*, one of the bethylid parasitoid of *H. hampei*.

Entomopathogenic Nematodes (EPNs) for control of H. hampei

The paper by Allard and Moore (1989) constitutes the first report of EPNs for *H. hampei* control. In laboratory experiments they demonstrated that a *Heterorhabditis* sp. (Rhabditida: Heterorhabditidae) causes mortality in adults and larvae of *H. hampei*, and suggested its use mainly against populations of the beetles attacking fallen berries on the soil. Later, Molina and Lopez (2002) demonstrated in the laboratory that *H. bacteriophora* Poinar and *Steinernema feltiae* (Filipjev) (Rhabditida: Steinernematidae) have the ability to locate, penetrate and attack *H. hampei* inside coffee berries, causing high levels of mortality inside ripe berries.

More recently, Lara et al. (2004) found that the two nematode species not only can locate the hosts in the berries, but also are able to reproduce inside the immature stages and adults of *H. hampei*, thus having the potential to reduce pest populations in the field.

In addition to *H. bacteriophora* and *S. feltiae*, Castillo et al. (2002) lately discovered *Sphaerulariopsis* sp. nov. (Tylenchida: Sphaerularioidea) in Mexico. This nematode species attacks immature and adult stages of *H. hampei* in the field. Poinar et al. (2004) re-classified it as *Metaparasitylenchus hypothenemi* sp. n. (Allantonematidae: Nematoda). The nematode does not cause high mortality of *H. hampei* stages, however, it substantially reduces the fecundity of females (Poinar et al., 2004).

Entomopathogenic Fungi (EPF) for control of H. hampei

Control of *H. hampei* using EPFs, and specifically *B. bassiana*, has been reviewed in great detail by Damon (2000). In more recent experiments in Colombia *B. bassiana* effectively controlled *H. hampei* in the field using a dose of $1x\ 10^{10}$ to $1 x\ 10^{12}$ spores per coffee tree (Posada, 1998). Moreover, efforts have been undertaken to design more realistic bioassays to quantify the effectiveness of *B. bassiana* as *H. hampei* biocontrol agent. For instance Posada et al. (2002), using a leaf spraying bioassay, concluded that not only the virulence of the isolate has to be taken into account, but also factors like formulation, number of drops per leaf surface and number of spores per drop of solution are critical features that might affect the degree in *H. hampei* mortality. However, a concentration like the one used by Posada (1998) is at present economically not feasible. An enhancement of the virulence of the

fungus, however, would permit the use of a reduced dose rate. Recent studies at CENICAFE focus on the genetic diversity of *B. bassiana* with the aim of future genetic modifications (Gongora, 2005), building on previous successful experiences with *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) (Rodríguez and Góngora, 2005). However a great deal of work would need to be done before releasing a genetically modified fungus into the field. The main obstacle is that the above fungal species have wide host ranges and their biology and ecology in the coffee environment is almost completely unknown. To our knowledge, so far there are no convincing long-term studies that show the economic feasibility and the practical use of EPF at farm level. In a study on the adoption of *H. hampei* IPM components in the Antioquia department in Colombia, 50.6% of the interviewed farmers had used EPFs for *H. hampei* control in the past, yet 71.1% of them had recently stopped using them because of, among others, their lack of efficacy (Mejia and Lopez, 2002).

Implications for *H. hampei* IPM

Cultural control of H. hampei*: the importance of post harvest management*

A complete removal of all ripe berries after the harvest and during the inter-harvest period is an important control measure as it reduces vital sources of *H. hampei* re-infestations. Rigorous collection of berries from the trees and from the ground, termed in Colombia the 'Re-Re' strategy (for 'Recoleccion' and 'Repase', i.e. harvesting of berries and immediately thorough re-collection of remaining berries in the same field), can substantially reduce infestations in the field because ripe and dry berries harbouring *H. hampei* are removed, thereby reducing the source population of

the beetles in the plantation (Bustillo et al., 1998). In addition to such a rigorous removal of ripe coffee berries, a careful handling of *H. hampei*-infested berries after the harvest must be implemented, as this will prevent emergence of *H. hampei* females from infested berries and their return to the plantation. Castro et al. (1998) calculated that during harvest between 66 and 74% of the *H. hampei* population present in the plantation ends up in the processing area, and, if the coffee is not properly handled, a high proportion of the pest will return to the plantation to re-infest new berries.

In Colombia several post-harvest control strategies have been developed and since 1998 implemented in a participatory manner with small-scale coffee growers (Baker et al., 2002). Fibre bags with a one mm mesh size are used, instead of plastic containers, to harbour the freshly picked coffee berries, thereby preventing the escape of *H. hampei* females (Bustillo et al., 1998). This also allows air exchange that reduces the relative humidity inside the bags (Bustillo et al., 1998) since high levels of relative humidity are a strong trigger for the emergence of *H. hampei* (Baker et al., 1992). Containers with freshly harvested berries are covered in the washing stations with oil-smeared plastic covers to trap emerging *H. hampei* females (Bustillo et al., 1998). In a participatory research program with 115 small-scale coffee growers in Colombia, Salazar et al. (2003) during one harvest period recorded the mean number of *H. hampei* females trapped in such a manner ranging from 1,576 to 20,266 per m^2 of lid. After the pulping the coffee beans are washed and the remaining pulp is usually composted. To prevent an escape of *H. hampei* females that have survived the pulping process, the piles of pulp are sprayed with *B. bassiana* (Bustillo et al., 1998).

Modification of the coffee dryers through addition of a muslin cover, helps to prevent the return of *H. hampei* to the plantation (Velez, 2000; Velez et al., 2002).

Though these strategies of cultural and post-harvest control of *H. hampei* are extremely laborious and consequently very costly, especially for small-scale coffee growers (Baker, 1999), a recent study by Aristizabal et al. (2002) on the adoption of IPM strategies against *H. hampei* in Colombia reported that with an adoption rate of 89%, "Re-Re" is by far the most frequently implemented control method, followed by post-harvest control with 40%.

The problem, however, with the emphasis on crop sanitation using "Re-Re" is that biological control agents are removed along with *H. hampei*, thus reducing their effect and this therefore effectively removes a potential central pillar of an IPM strategy. In practice, crop sanitation is mostly carried out as a routine measure rather than based on a threshold decision because of the difficulty of accurately sampling pest levels on tree and ground (Baker, 1999). It seems that many farmers have arrived at a combination of sanitation and spraying that is at best only a rudimentary form of IPM. With the advent of high-density plantings (up to 10,000 trees/ha) the human contamination during spraying is high, though we know of no published studies on this aspect.

Traps as an IPM component

In 1991, a study by Mendoza-Mora documented for the first time that a 1:1 mixture of methanol and ethanol could act as an attractant to coffee berry borer females (Mendoza-Mora, 1991). Subsequently, Mathieu et al. (1997) showed that visual stimuli are also important in host location by *H. hampei*. They tested white and

red multi funnel traps (Lindgren, 1983), baited with a 1:1 mixture of ethanol and methanol at three dose rates (i.e. 0.5, 1.5 and 20 g/day) and concluded that red traps baited with low doses are more attractive to *H. hampei* females. They were able to catch 45% of the initially released *H. hampei* females. The volatile composition of *C. arabica* berries of different ages is highly dominated by different alcohols (Ortiz et al. 2004). Using ethanol: methanol baited traps, Dufour et al. (1999) conducted field studies in El Salvador and achieved a reduction in *H. hampei* infestations by 34.8% which subsequently led to the development of the commercial *H. hampei* trap BROCAP® (Dufour et al., 2001). Its validation under field conditions in commercial coffee plantations in El Salvador resulted in reductions in infestations levels of up to 80% (Dufour et al., 2004).

Some issues surrounding coffee, *H. hampei,* IPM and sustainability

We believe that in recent years IPM in coffee has lost ground to 'sustainability'. The latter covers matters related with farming and labour relations, marketing, water conservation and other things that are often subsumed under economic, environmental and social subheadings. With this concept of sustainability, IPM becomes merely one element among many others that the farmer has to comply with to achieve certifications for their produce (e.g. Fair Trade, Rainforest Alliance schemes). Such certifications in general can offer coffee growers (large plantation owners as well as small-scale farmers) significant economic benefits through higher prices.

Sustainability has undeniably become a force that has tackled some major issues and through certification brands, has brought these issues into the public domain. In this sense the advent of sustainable schemes can be seen as a way to increase IPM implementation. At a practical level though, there is a risk that available IPM information is incorporated into a farm management plan for the purposes of certification, and that this then becomes regarded as standard practice, even though the IPM schemes in question, for instance the use of EPFs and augmentative mass releases of the bethylid parasitoids of *H. hampei* (Fischer-Worrung et al., 2001), are still in a research and development stage. On the other hand, sustainable schemes stress the safety aspects of pest control such as restrictions on the use of the most toxic compounds and this should lead to increased demands for biological control solutions.

These two aspects together suggest that researchers need to review the current recommendations for sustainable schemes. This should include i) an evaluation of the efficacy of some of the current recommendations to avoid misconceptions and inconsistencies in some of the advice provided, and ii) formulate a new research agenda to find answers to enduring problems in coffee such as *H. hampei*. Thus we believe that there is an urgent need to develop clear and effective IPM-related guidelines for the increasing number of certification bodies to whom IPM of pests is but one of many tasks. However, IPM scientists also need to consider broadening their research mandates to include sustainability themes, such as insect indicators for biodiversity, or long-term studies of organic farms. Another future challenge will be to bring in small-scale coffee growers into the sustainable domain, and this should be seen as a chance to re-evaluate the role of IPM for the underprivileged sector. The

latter implies a necessity to make IPM practices easier to implement and more realistic at the farm level.

During recent years, research on control of *H. hampei*, and the development and implementation of IPM programs, in general has not advanced markedly and reasons may involve a shortage of funds due to the coffee price crisis and the advent of the sustainable coffee schemes that have attracted much attention from donors who previously funded IPM research. Thus we believe it is time for a coordinated approach involving all parties concerned by the coffee berry borer problem, i.e. IPM researchers, certification bodies, the coffee industry, extensionists and of course the coffee farmers themselves, to re-assess the role of research and how to employ it to best effect in the future.

CHAPTER 2

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

Abstract

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

Keywords: Coffee berry borer; *Hypothenemus hampei*; *Phymastichus coffea*; Oarasitism of adults; Field conditions; Colombia.

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Introduction

The Coffee Berry Borer (CBB) *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae) is the most important coffee pest worldwide (Le Pelley, 1968; Damon, 2000). CBB 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, CBB was first recorded in the southern part of the country in August 1988 (Bustillo et al., 1998). To date CBB is widespread throughout all coffee growing regions of Colombia and is 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 CBB infestation can alter this ratio up to > 17:1 (Baker et al., 2002).

CBB females 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 only by one CBB 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 CBB 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 CBB (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 the sufficient amount of dry matter content for the development of her offspring (Alonzo, 1984; Ruiz, 1996).

Initially Colombian coffee growers tried to combat CBB infestations predominantly through use of broad-spectrum insecticides. However, growing environmental concerns and increasing problems with insecticide resistance in CBB (Gongora et al., 2001) stimulated the search for environmentally more friendly control strategies against CBB in Colombia. As an exotic outbreak pest in South America, a classical biological control approach was pursued. Initially two larval-pupal ectoparasitoids of CBB, 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 1980ies (Baker, 1999). They were first tested under laboratory conditions and subsequently released in the field (Benavides et al., 1994; Portilla and Bustillo, 1995). Although both parasitoids successfully established in Colombia their impact on field populations of CBB has been rather limited (Quintero et al., 1998).

In 1996 a third parasitoid of CBB, the eulophid *Phymastichus coffea* LaSalle, was introduced to Colombia. *P. coffea* was found in Togo in 1987 (Borbón, 1989) and described by LaSalle (1990). It is a gregarious endoparasitoid of CBB females (Borbón, 1989), and usually one male and one female *P. coffea* develop inside each host (Lopez and Moore, 1998). After being parasitized, the mobility of the CBB 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 lifespan 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 parasioid 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 CBB 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 1,400 m; 21.4°C mean annual temperature; 2,700 mm precipitation/year; 80% mean relative humidity) near Chinchiná, Colombia. A five year old *Coffea arabica* (L.) cv. Colombia crop 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 previously not been treated with synthetic insecticides nor had parasitoids of CBB been previously released there. However, 'Re-Re' the cultural control practice against CBB 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

CBB females 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, CBB of mixed age were collected in the rearing, transferred to plastic boxes filled with staple paper, and then brought to the field.

P. coffea adults used in the experiments also originated from the CENICAFE rearing unit. They are mass-produced on CBB-infested parchment beans, following the protocol developed by Orozco (2002). In our study, immediately after emergence from the CBB 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.

CBB artificial infestations 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 three different periods after blossoming, i.e. 90, 150 and 210 days, mimicking the infestation pattern of CBB in berries of different ages (Salazar et al., 1993). During these periods the mean dry matter content of coffee berries are 0.0224, 0.2689 and 0.5149 g in 90, 150 and 210 days old berries, respectively (Salazar et al., 1994). The selected branches were then covered with an entomological sleeve, and artificially infested with 250 CBB females per branch, following the methodology described by Villalba et al. (1995). After 24 hours the sleeves were removed, assuring a 100% CBB infestation of the berries. The parasitoids were released around the selected branch in a ratio of 1:1 to the number of CBB infested berries per branch, i.e. 50 *P. coffea* per tree. For each age of CBB infestation they were released at three different intervals, i.e. one, five and nine days after the artificial infestation of the branches with CBB. Consequently nine treatments based on the combinations of the age of the berries and the time of the *P. coffea* releases after the initial CBB infestation 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 CBB inside the berry was recorded. According to Bustillo et al. (1998) the positions of the CBB female in the coffee berry are defined as: position A, when CBB is starting the colonization of a new berry and the penetration of the exocarp begins; position B, when CBB 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 CBB has produced a gallery in the endosperm, and one or more of its immature stages are found inside the gallery (fig. 1).

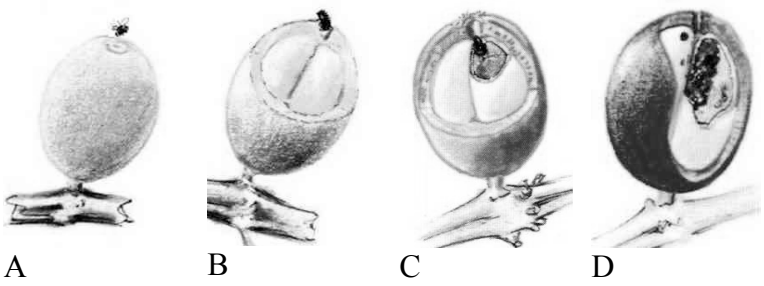

Figure 1. Positions of coffee berry borer *Hypothenemus hampei* in coffee berries (Bustillo et al., 1998; drawing by Gonzalo Hoyos, CENICAFE).

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 CBB.

Statistical analysis

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

Results

Levels of CBB parasitism by *P. coffea* were significantly affected by the age of the berries at the time of CBB infestations, the position of CBB inside the berries, and the age by position interaction (table 1). The time of the *P. coffea* releases after artificial infestation of berries with CBB did not affect parasitism levels. Similarly, the interaction between time of parasitoid release and age of the berries at the time of CBB infestations had no effect on rates of parasitism. However, the interaction of time of parasitoid release and position of CBB in the berries, as well as the three-way interaction between berry age at the time of CBB infestations, parasitoid release time and the position of CBB in the berry significantly affected rates of CBB parasitism by *P. coffea* (table 1). Consequently, parasitism levels were compared at a given position of CBB 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 CBB infestation, as well as at a given age of the coffee berries at the time of CBB infestations, for a given position of CBB in the berries across the different times of *P. coffea* releases (table 2).

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 days 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	-	-

In 90 days old berries the proportion of CBB 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 CBB adults found in position A of 150 and 210 days old berries. In 90 days 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 CBB infestation (table 2).

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 (A, B, C, D) in 90, 150 and 210 days old coffee berries.

Position of CBB in the berries	Time of *P. coffea* release (days)	CBB found parasitized in each position (% ± SE)		
		90	150	210
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).

For the same CBB position, significantly lower levels of parasitism were recorded in 150 and 210 days old berries one day, and in 210 days old berries also nine days after the parasitoid releases compared to 90 days old berries. Significantly fewer CBB in position B (1.8%) were parasitized in 210 days old berries nine days after the *P. coffea* releases compared to the two earlier parasitoid releases (table 2). For CBB in position C highest levels of parasitism were recorded in 150 compared to 90 and 210 days old berries independent of the *P. coffea* release dates (table 2). With increasing time between artificial CBB infestations and *P. coffea* releases, parasitism significantly decreased in 90 and 150 days old berries. No or only very low parasitism levels were recorded in CBB 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 days old berries, and one day after a *P. coffea* release also in 90 days old coffee berries (table 3). Additionally, percentages of parasitism for a given age of the berries at the time of CBB infestations, and for a given *P. coffea* release were compared across the different positions of CBB inside the coffee berries. In 90 days 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 days 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 days old berries significantly highest parasitism level was always recorded in CBB found in position C (table 3). For CBB in position D, low levels of parasitism were only recorded in 150 days old berries nine days after the *P. coffea* release and in 210 days old coffee berries independent of the time of the releases of the parasitoids (table 3).

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 days old coffee berries.

Age of berries (infestation)	Time of *P. coffea* release (days)	Total parasitism (% ± SE)	CBB found parasitized in each position (% ± SE)			
			A	B	C	D
90 days	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 days	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 days	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).

Discussion

Levels of CBB parasitism 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 CBB 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 CBB female, i.e. positions A and B (see fig. 1), and subsequent oviposition, i.e. position D, under field conditions in Colombia are 70 and 5 days, for 90 and 210 days old berries, respectively (Ruiz, 1996). We recorded highest levels of parasitism in beetles found in position C of 150 days old berries independent of the time of the parasitoid releases. At this time parasitized CBB adults found in position C had just begun damaging the endosperm and in no case oviposition chambers were observed. CBB females stop ovipositing and their mobility is impaired after parasitization by *P. coffea* (Feldhege, 1992; Infante et al., 1994). In 90 days old berries when *P. coffea* was released one day after the artificial CBB infestation around 60% of the parasitized beetles were found in position B. Yet when the parasitoids were released five or nine days after the CBB infestation, highest levels of parasitism were recorded in CBB 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* L. (Coleoptera: Scolytidae) and *Tomicobia seitneri* (Ruschka) (Hymenoptera: Pteromalidae), where parasitized beetles continued to bore into the bark (Sachtleben, 1952). Likewise Feldhege (1992) observed that CBB 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 CBB females in positions C or D (Borbón, 1989; Infante et al., 1994; Lopez and Moore, 1998). Thus the high levels of parasitism recorded in 90 and 150 days old berries might be due to the long time CBB 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, CBB 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 CBB in 210 days 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, CBB females 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 CBB in position D in 210 days old coffee berries.

The results of this study showed that *P. coffea* is a promising biological control candidate for CBB 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. CBB populations tend to remain inside coffee berries and reproduce at a higher rate during the dryer periods, whereas reproduction decreases and migration and subsequent colonization of new berries increase during the rainy season (Baker et al., 1994; Bustillo, 2002). Generally CBB populations start colonising coffee berries between 100 to 150 days after blossoming (Salazar et al., 1993). Since CBB 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 crypto-parasitoids *C. stephanoderis* and *P. nasuta* should be released to complement *P. coffea*. Both larval-pupal parasitoids are capable of parasitizing CBB 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 (Damon, 2000; Baker, 1999).

The parasitoid-host ratio used in releases in this study was high and could be too costly to implement in an area-wide management program. However, attempts are underway to improve the CBB rearing, a crucial factor for the parasitoid production, through the development of an artificial diet for CBB (Portilla, 1999). Moreover, 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 CBB. In summary, our data clearly underlines the great potential of *P. coffea* for biological control of CBB in Colombia and other coffee growing countries of South and Central America.

CHAPTER 3

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

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 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 number of *P. coffea* larvae per female host were assessed ten 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 2 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.

Keywords: Coffee; Hymenoptera; Eulophidae; Coleoptera; Curculionidae; Scolytinae; Biological Control; Dry Matter Content; Plant Effects.

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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 (Borbon-Martinez, 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 pre-oviposition period, whereas Feldhege (1992) reported pre-oviposition periods between 5 min and 4 h, with 20 min as the most frequent duration. Female *P. coffea* oviposit 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 and 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 one 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 and 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 and 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 (Islam and Copland, 2000; Sirot et al., 1997)] or the quality of hosts encountered (Goubault et al., 2004; Waage and Godfray, 1985), 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 and Visser, 1990). Under natural conditions, intraspecific competition is predicted to influence clutch size (Visser and 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; 1,400 m above sea level; 21.4 °C mean annual temperature; 2,700 mm precipitation/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 ºC, 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 provided 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 *Coffea arabica* (L.) cv. Colombia crop 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, 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 x magnification), 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. Pair-wise 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 in 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 releases of the parasitoids (Figure 1).

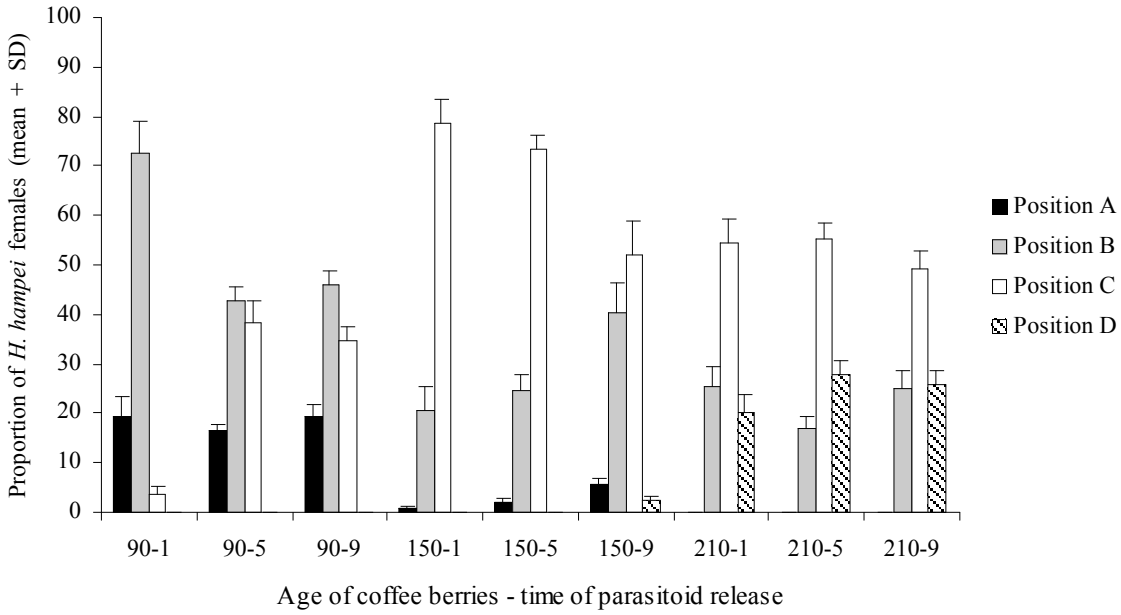

Figure 1. Proportion of *Hypothenemus hampei* females (mean + SD) found in 90-, 150-, and 210-day-old coffee berries following releases of *Phymastichus coffea* 1, 5, and 9 days after artificial infestation of the coffee berries with the beetles; positions A – D refer to the depth of penetration of *H. hampei* into the coffee berries (for details see text).

All three variables, i.e., age of the coffee berries/age of artificial infestation with *H. hampei* (χ^2 = 46.90, d.f. = 2, P<0.0001), time of parasitoid release (χ^2 = 223.22, d.f. = 2, P<0.0001), and position of the beetles inside the berries (χ^2 = 13.01, 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 1-9 days after coffee berry infestations with *H. hampei* (Figure 2).

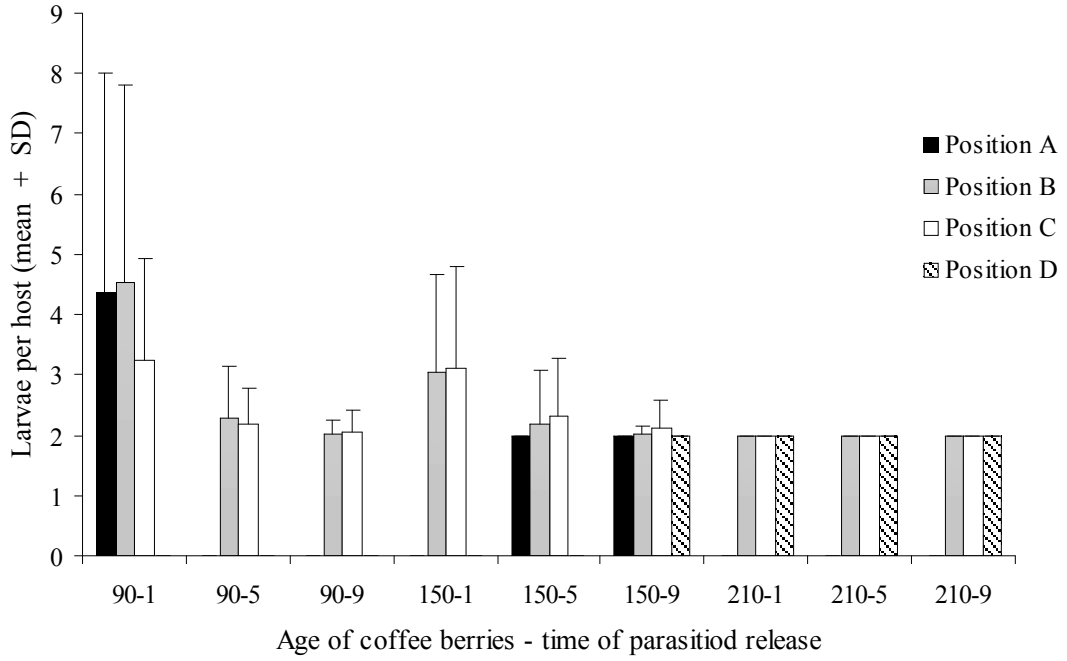

Figure 2. Number of *Phymastichus coffea* larvae per host (mean + SD) found in *Hypothenemus hampei* adults attacking 90-, 150-, and 210-day-old coffee berries following parasitoid releases 1, 5, and 9 days after artificial infestation of the coffee berries; positions A – D refer to the depth of penetration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

The number of parasitoid larvae per *H. hampei* in positions A and B was significantly higher than in positions C and D (χ^2 = 438.97, 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 (χ^2 = 6.71, d.f. = 1, P = 0.0096) and positions B and C (χ^2 = 9.76, d.f. = 1, P = 0.0018). However, no significant differences were found between positions A and B (χ^2 = 1.59, d.f. = 1, P = 0.2074), A and D (χ^2 = 1.41, d.f. = 1, P = 0.2347), B and D (χ^2 = 0.20, d.f. = 1, P = 0.6544), and C and D (χ^2 = 0.83, 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 one day after the *H. hampei* infestation in this berry age class, high numbers of parasitoid larvae were found in hosts in position A (4.4), B (4.5), and C (3.2) (Figure 2). When parasitoids were released five and nine days after the *H. hampei* infestation, however, no hosts were found in positions A and D, and the number of parasitoid larvae per host in the positions B and C were 2.3 and 2.2, respectively.

Likewise, releases of *P. coffea* one and five days after the artificial infestation of 150-day-old coffee berries by *H. hampei* resulted in high numbers of parasitoid larvae per host female in 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 one and five days after the artificial infestation with *H. hampei* in 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).

Table 1. Results of LSMEANS pair-wise 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

Effect	d.f.	χ^2	P > χ^2
Age of coffee berries/ infestation with *H. hampei*			
90 days / 150 day	1	15.82	<0.0001
90 days / 210 days	1	44.69	<0.0001
150 days / 210 days	1	19.88	<0.0001
Time of *P. coffea* release			
1 day / 5 days	1	127.30	<0.0001
1 day / 9 days	1	180.55	<0.0001
5 days / 9 days	1	4.57	0.0325

Discussion

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 and Visser, 1990), food availability (Harvey et al., 2001), and the physiology of the female parasitoid, including its egg load (Babendreier and 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 superparasitisim 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 effects 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, 1996; Jaramillo et al., 2005).

In this study, superparasitism, either self or conspecific, was recorded when *P. coffea* were released at a time when *H. hampei* had just commenced penetrating the coffee berries (positions A and B), and were 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 and 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 and 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 in 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, 1996). 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., in position D, were found in position B (J 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 (Infante et al., 2005; Lauzière et al., 2000), 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 and 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 and 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 meters' 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 *H. hampei* by *P. coffea* under fields conditions.

An additional factor that might have contributed to the extent of superparasitism by *P. coffea* is the host/parasitoid release ratio. Presently little is known on optimal host-parasitoid release ratios for *P. coffea* and *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 and Visser, 1990), because the decision to stay longer in the patch and superparasitize is strongly influenced by the presence of competing conspecifics (van Alphen and Vet, 1985), which eventually affects clutch sizes (Visser and 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 *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 *H. hampei*-infested branch. Thus, our results are in line with expectations of Visser and Rosenheim (1998).

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

CHAPTER 4

Parasitoids of the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae) in Kenya: a two-year exploration*

Abstract

Cephalonomia stephanoderis and *Prorops nasuta* are two of the three parasitoids of African origin that have been introduced to coffee producing areas of the Americas as biological control agents of the coffee berry borer (CBB) *Hypothenemus hampei* (Coleoptera: Curculionidae). Both bethylid 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 *P. nasuta* to be the most important, effective, and dominant CBB parasitoid, with CBB-infested coffee berries that have fallen to the ground being the main source of CBB natural enemies. The design and field use of a tent-like structure to place CBB-infested coffee berries after they are harvested as part of the sanitation of the crop within the cultural control component of the CBB IPM, which allows the emergence of parasitoids but not of the pest, is discussed. This structure could serve to enhance CBB biological control by *C. stephanoderis* and *P. nasuta* in the Americas.

Keywords*:* *Hyopthenemus hampei*; Coffee Berry Borer; Coffee; Biological Control; Parasitoid; *Prorops nasuta*; Hyperparasitoid; IPM.

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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 60 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 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 this pest.

Previous explorations for natural enemies of the coffee berry borer in Africa have revealed the presence of various parasitoids, including the braconid *Heterospilus coffeicola* Schmiedeknecht, which was reported by Hargreaves (1926) in Uganda. However, no viable rearing protocols have been developed for this wasp, limiting its use in biological control programs (Murphy et al., 2001). In addition, two bethylids have been reported: *Cephalonomia stephanoderis* Betrem and *Prorops nasuta* Waterston originating from the Ivory Coast (Ticheler, 1961) and Uganda (Hempel, 1934), respectively. These bethylids are larval-pupal ectoparasitoids of the CBB and usually prey on females and eggs (Perez-Lachaud, 2002; Infante et al., 2005). A

fourth parasitoid species, the eulophid *Phymastichus coffea* LaSalle was discovered in Togo in 1987 (Borbón-Martinez, 1989).

C. stephanoderis, *P. nasuta* and *P. coffea* have been introduced to coffee growing areas in the American continent (Barrera et al., 1990; Baker, 1999) and in India (Duque and Baker, 2002), and are presently being used in an integrated pest management (IPM) program involving the combined use of parasitoids, entomopathogenic fungi (e.g., *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales)), trapping, and cultural control.

Although, *C. stephanoderis* and *P. nasuta* became established in the countries where they were released (e.g., Mexico, Colombia and India), their impact on *H. hampei* field populations has been limited to 5% or less (Quintero et al., 1998; Baker, 1999; Infante et al., 2001).

The cultural control component of the CBB IPM program 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' i.e. harvesting of berries and immediately thorough re-collection of remaining berries in the same field), can substantially reduce infestations 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, 2002). In spite of its cost, an analysis of the different CBB integrated pest management options in Colombia concluded that the cultural control is the most important and widely used component (Aristizabal et al., 2002; Benavides et al., 2002).

This study presents the results of a 2-year search for new natural enemies of the coffee berry borer in Kenya as part of an attempt to develop more efficient, economically feasible, and environmentally sustainable strategies to control the CBB.

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, 1,510 meters above sea level [masl]). A plot of 2,000 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) (Figure 1).

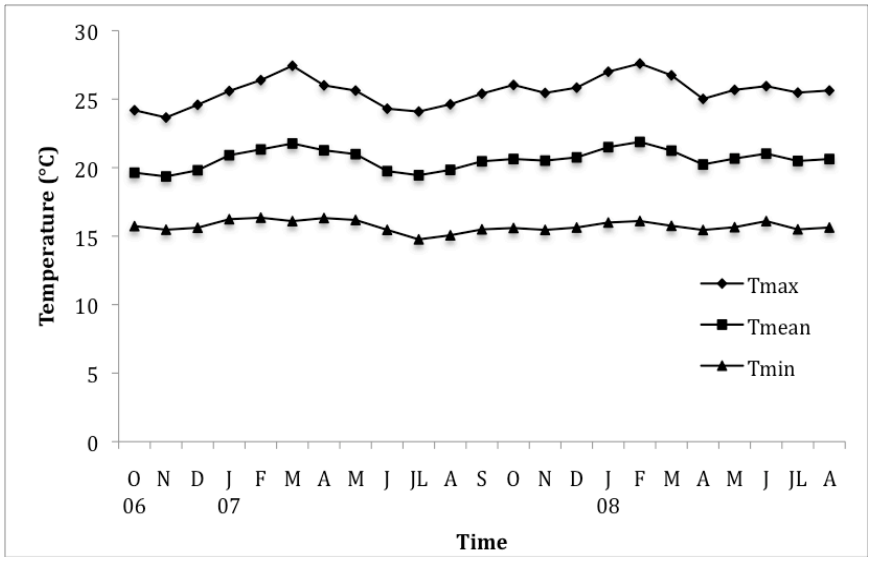


Figure 1. Mean monthly temperatures for the area of study in Western Kenya.

Samples of CBB-infested coffee berries both from the tree and the ground, i.e. the litter strata, were collected at 2-3 weeks intervals for 2006 and 2007 and weekly in 2008. Between 100-150 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 minutes, rinsing with tap water, then dipping in a 2% sodium hypochlorite solution for 10 minutes, 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 x 40 x 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., 2008), 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 three 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 two 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. Specimens were identified by taxonomists at the Systematic Entomology Laboratory, United States Department of Agriculture, Agricultural Research Service, in Belstville, Maryland, USA.

Statistical analyses

Data on emergence of parasitoids are presented separately for berries collected in the ground and in the tree. Descriptive analyses were carried out separately for data on emergence of parasitoids and for dissections of berries.

Differences in the parasitoids emerging from the field collected berries and the numbers of CBB immature stages and adults and the parasitoids and hyperparasitoid found during the dissections were analyzed by analysis of variance (ANOVA), using the PROC MIXED procedure of SAS (SAS, 1999). PROC MIXED procedure was also used to analyse the difference in the emergence trend of parasitoids from year to

year. An *F* test was used to test the significance of mean differences and least square mean (LSM) values were computed. The significance level was set at $P = 0.05$.

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

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

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

Results

Parasitoid species complex

Emergence of parasitoids was recorded in a total of 32,780 berries from the trees and 36,729 berries from the litter (ground) strata, collected from October 2006 to September 2008. An additional 3,842 berries were dissected for CBB life stages and natural enemies. In total 333 parasitoids/ hyperparasitoids were collected from berries harvested from the trees and 10,409 specimens emerged from the ground strata samples. All of these parasitoids/ hyperparasitoids are considered to be associated with CBB (Table 1).

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

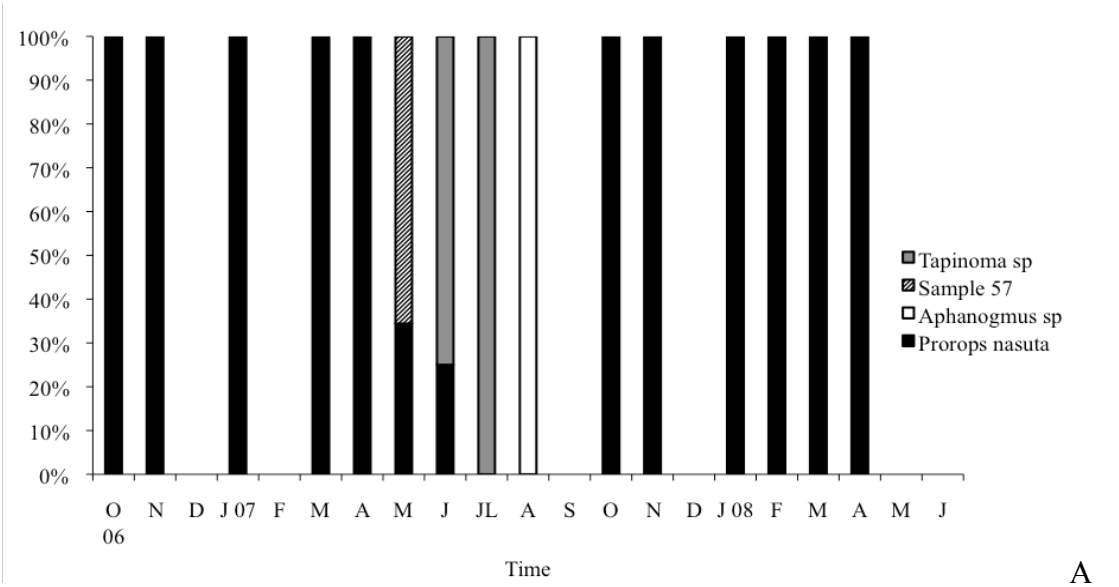
From the coffee berries that were collected on the ground the most dominant parasitoid was again *P. nasuta* accounting for 82.2% of the total emergence, followed by its hyperparasitoid *Aphanogmus* sp (12.7%), sample 57 (4.4%), sample 54 (0.1%), *P. coffea* (0.1%), *P.* near *schedli* (0.1%), *C. stephanoderis*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

Table 1. Most abundant species recovered from coffee berries collected in the ground strata and from coffee trees.

Species	Habit
Hymenoptera	
Bethylidae	
Cephalonomia stephanoderis	Primary CBB parasitoid (Ticheler, 1961)
Prorops nasuta	Primary CBB parasitoid (Hargreaves, 1926)
Goniozus sp	Possible new CBB parasitoid
Ceraphronidae	
Aphanogmus sp	*P. nasuta* hyperparasitoid (Jaramillo and Vega, 2008)
Eulophidae	
Phymastichus coffea	Primary CBB parasitoid (Borbon-Martinez, 1989)
Formicidae	
Tapinoma sp	Possible CBB predator
Pteromalidae	
Pachycrepoideus near *schedli*	Possible CBB parasitoid (OILB 1971)
Others	
Sample 54	Under identification
Sample 57	Under identification

Seasonal dynamics and abundance of the parasitoid species

Figure 2 (A and B) show the dynamics of the CBB parasitoid complex over time for the trees and ground strata. The dominant species during the entire investigation period 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.



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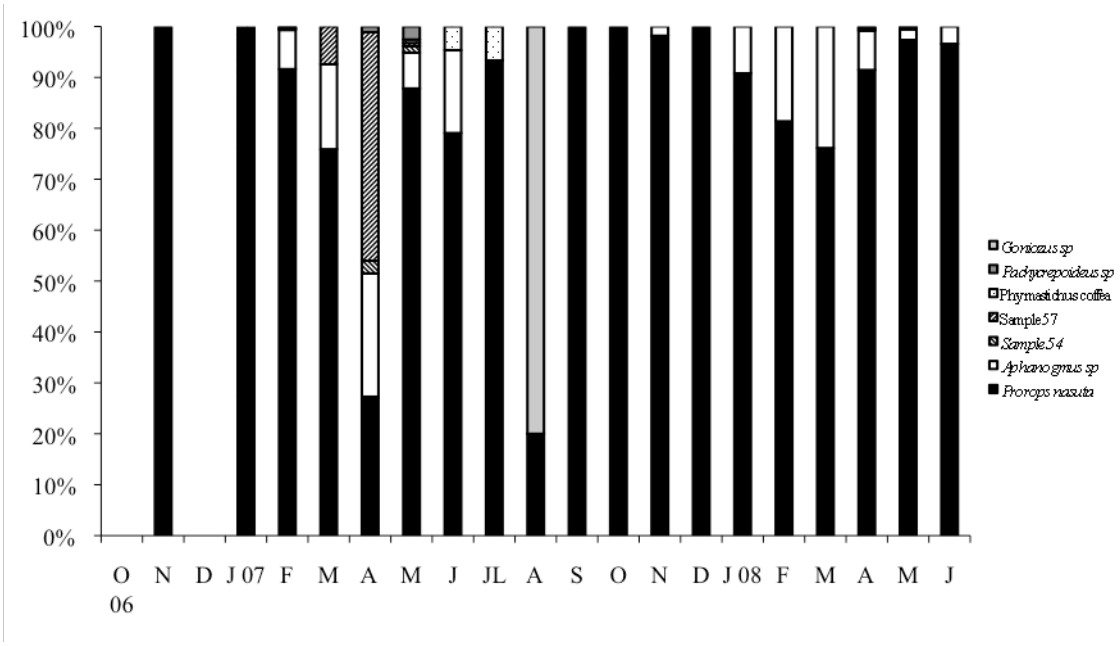


B

Figure 2. Temporal dynamics of the CBB parasitoid complex recorded from coffee berries collected in/on a) the trees, b) the ground.

Abundance of P. nasuta *and* Aphanogmus sp *from ground vs. tree: emergence*

The numbers of parasitoids/ hyperparasitoids recovered over time from tree and ground berries differed significantly ($F = 75.06$, $P < 0.0001$). The strata where the berries were collected, the date of collection and the interaction strata by date also significantly affected the numbers of emerged *P. nasuta* ($F = 120.36$, $P < 0.0001$) ($F = 21.23$, $P < 0.0001$) ($F = 20.80$, $P < 0.0001$), respectively, and *Aphanogmus* sp ($F = 42.19$, $P < 0.0001$) ($F = 10.78$, $P < 0.0001$) ($F = 10.54$, $P < 0.0001$), respectively.

In general, the emergence of *P. nasuta* started to increase from January of each year and peaked during March for ground berries in 2007 and in 2008 a second peak of emergence was recorded during June (Figure 3). For *A. goniozi*, emergence started one month later than it its presumed host *P. nasuta*, with peak emergence in April (Figure 3). Very few parasitoids/ hyperparasitoids were recorded until October. Subsequently, the emergences started to pick up again with 4 to 8 and 3 to 8 parasitoid and hyperarasitoid individuals, respectively, emerging per day during October, November and December 2007; thereafter the numbers of parasitoids/ hyperparasitoids recovered started to increase again. No significant differences were found between the emergence tendency of *P. nasuta* and *Aphanogmus* sp between 2007 and 2008 for ground berries ($F = 0.01$, $P = 0.9506$) ($F = 1.18$, $P = 0.2781$), respectively. Although the relative seasonal abundance of *P. nasuta* did not differ between the years, the numbers of emerged individuals during February, March and June 2007 and 2008 varied significantly ($F = 21.34$, $P = < 0.0001$).

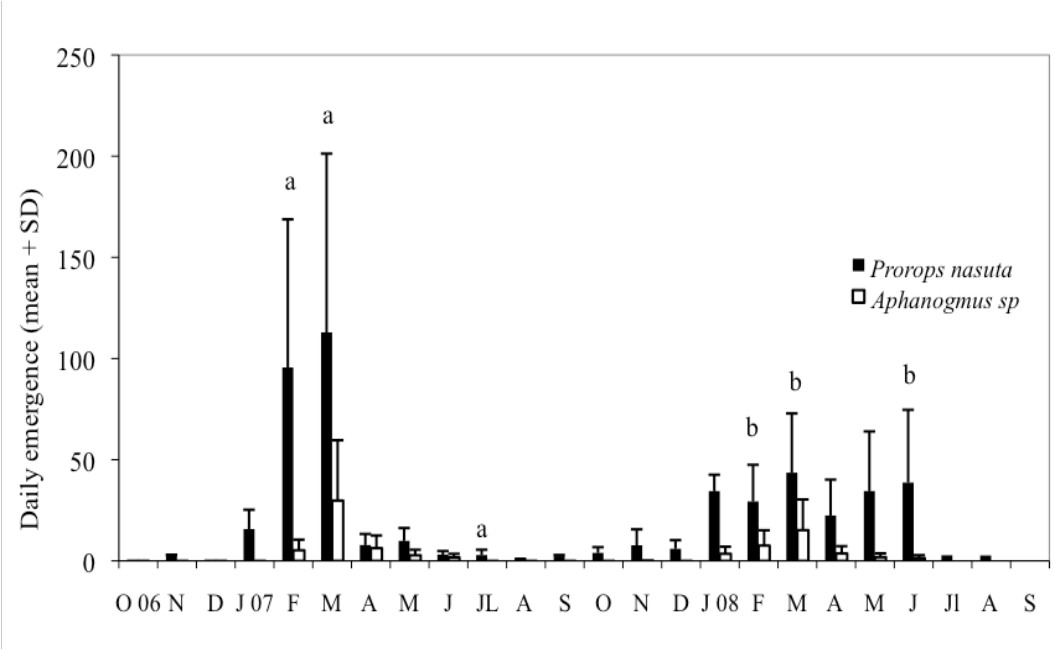


Figure 3. Daily emergence (mean ± SD) of *P. nasuta* and its hyperparasitoid *A. goniozi* from coffee berries collected from the ground strata. Emergence (mean ± SD) between a given month for 2007 and 2008, with the same letter are not significantly different (P= 0.05).

For the berries collected from trees *P. nasuta* emergence peaked in April, but additional peaks were also recorded during January, September, October and December 2007 (Figure 4). During 2008 one peak of emergence was recorded in February. In contrast, the number of *Aphanogmus* sp that emerged from berries collected from coffee trees was very low throughout the year, reaching only a maximum of up to 2 individuals a day during March 2007 (Figure 4). Similar to the ground collected berries, the emergence trend of *P. nasuta* and *Aphanogmus* sp between 2007 and 2008 did not differ significantly ($F = 0.13$, $P = 0.7220$) ($F = 1.31$, $P = 0.2530$), respectively. Despite the similarity in emergence trends between the

years, significantly more *P. nasuta* were recovered from ground berries in March and September 2007 compared 2008 ($F = 3.16$, $P = < 0.0001$).

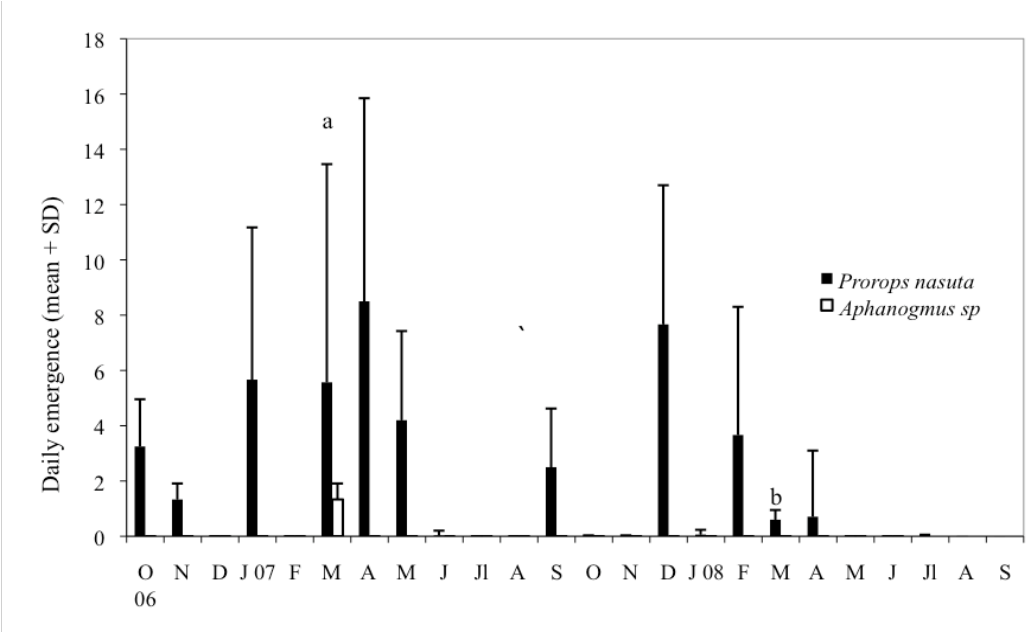


Figure 4. Daily emergence (mean ± SD) of *P. nasuta* and its hyperparasitoid *Aphanogmus* sp from coffee berries collected from the trees. Emergence (mean ± SD) between a given month for 2007 and 2008, with the same letter are not significantly different (P= 0.05).

Dissection of coffee berries

Dissections of the coffee berries were carried out only for the year 2008 after determining a trend in the emergence of the parasitoids. The place of collection of coffee berries (i.e., tree and ground) and the month of collection had a significant effect on the numbers of CBB immature stages ($F = 19.9$, $P < 0.0001$) ($F = 42.4$, $P < 0.0001$), total numbers of CBB females ($F = 19.3$, $P < 0.0001$) ($F = 31.6$, $P < 0.0001$), females found live ($F = 12.3$, $P < 0.0001$) ($F = 11.8$, $P < 0.0001$), dead ($F = 30.7$, $P <$

0.0001) ($F = 44.8$, $P < 0.0001$) and CBB female mortality ($F = 45.6$, $P < 0.0001$) ($F = 58.5$, $P < 0.0001$). Likewise the interaction place by month of collection was always significant except in the case of CBB immature stages ($F = 0.7$, $P = 0.6184$) and CBB female mortality ($F = 1.6$, $P = 0.1666$) (Figures 5-7).

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 (Figure 6a) and between 2.4 in May and 7.6 in March. CBB females that were found live during the dissections were higher in the berries sampled in the trees than in the ground (Figure 6b). The number of immature stages started to increase from February reaching the maximum number in June (Figure 5). The mortality of CBB was considerably higher during January, February and March and it started to decrease from April onwards (Figure 7). This reduction of CBB mortality coincides with the decrease in numbers of *P. nasuta* emerging from the coffee samples (Figure 4).

During the dissections, hardly any life stages of *P. nasuta* were found in tree berries. However, the place and month of collection of the coffee berries had an effect on the numbers for cocoons ($F = 16.6$, $P < 0.0001$), for *P. nasuta* live ($F = 12.9$, $P < 0.0001$), and for *P. nasuta* dead ($F = 26.4$, $P < 0.0001$). In ground berries, between 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 in berries collected on the ground and in the trees.

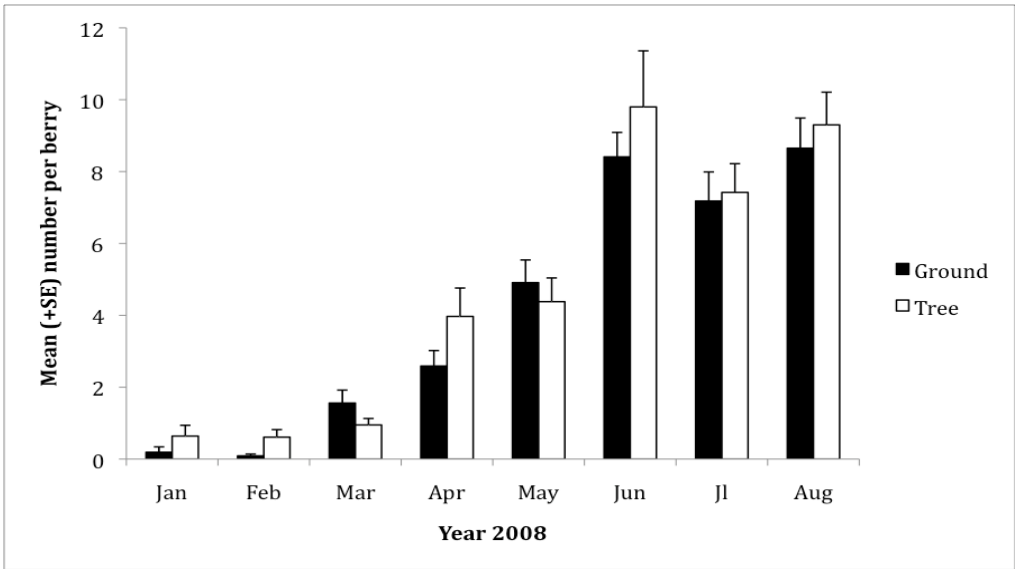
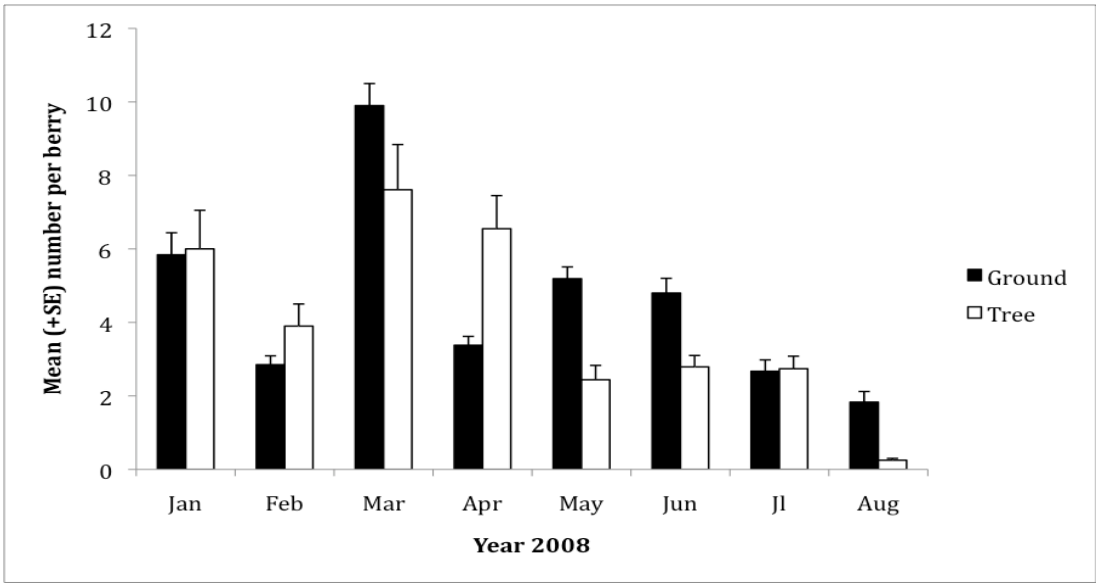


Figure 5. Mean (+SE) numbers of immature stages of *Hypothenemus hampei* inside coffee berries collected in the trees or on the ground over time.



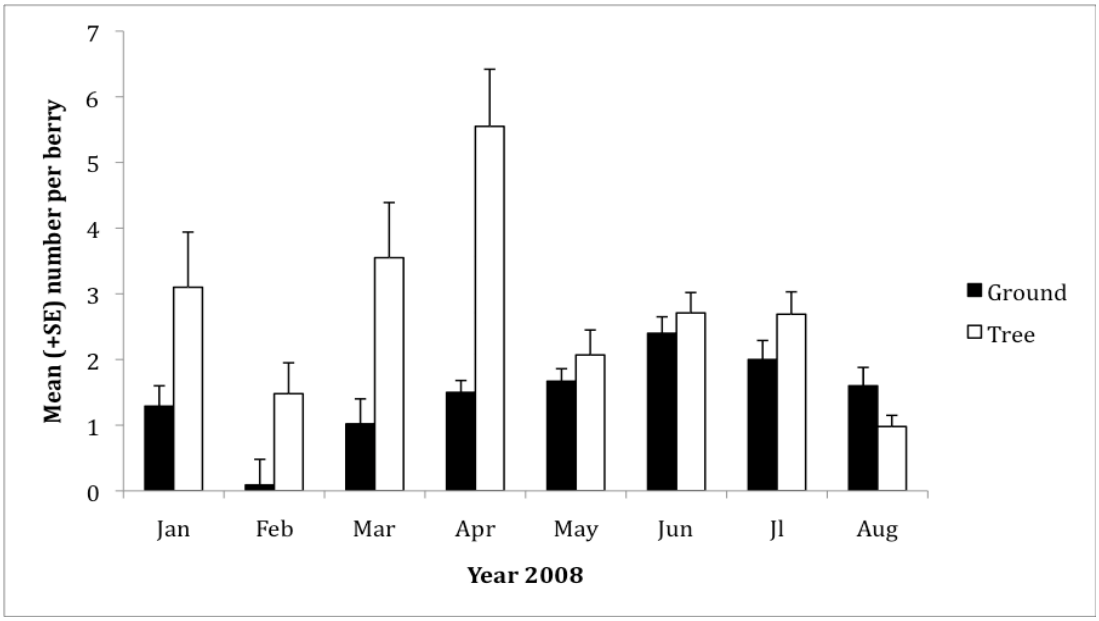


Figure 6. Mean (+SE) numbers of (a) *Hypothenemus hampei* females, and (b) *Hypothenemus hampei* live females inside coffee berries collected in the trees or on the ground over time.

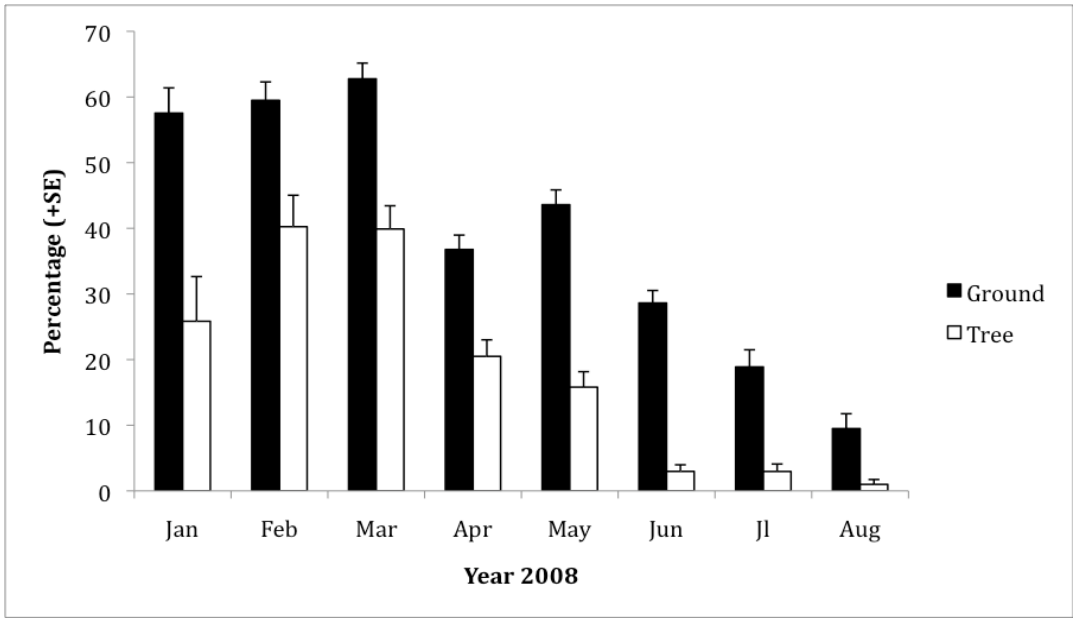


Figure 7. *Hypothenemus hampei* mortality in coffee berries collected from the trees or on the ground over time.

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.

Discussion

Two years of intensive sampling in the coffee growing region of Western Kenya revealed a surprisingly low diversity of parasitoids of the CBB. Apart from the bethylid parasitoid *P. nasuta* and its ceraphronid hyperparasitoid *Aphanogmus* sp (Jaramillo and Vega, 2008), all other known parasitoids of *H. hampei* were either absent or recorded in very low numbers. For instance the eulophid *P. coffea* was virtually nonexistent in our sampling area during the time 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). As a parasitoid of adult female beetles, *P. coffea* was originally considered a highly promising biological control agent (Gutierrez et al., 1998; Jaramillo et al., 2005) but so far, its impact on CBB populations in the countries where it was released has been rather limited. It was also striking that the braconid *H. coffeicola* was never found during our 2 years of sampling. In a similar study in neighboring Uganda, Hargreaves

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

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 2 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 to three *Aphanogmus* sp adults on average emerged from the host cocoons (Jaramillo and Vega 2008).

A possible 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 plantations were not shaded and were 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 (Perez-Lachaud et al., 2002; Perez-Lachaud et al., 2004; Batchelor et al., 2005; Batchelor et al., 2006). However, Batchelor et al. (2006) has recently suggested that *P. nasuta* should be the most effective biological control agent of the 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 our study, out of the 8,893 individual *P. nasuta* collected, 238 emerged from berries picked from the trees, whereas 8,655 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. van Emden, pers. comm.). Yet, two strikingly similar examples to CBB and *P. nasuta* has been reported for fruit flies and the horse chestnut leafminer, *Cameraria ohridella* Deschka and Dimic (Lepidoptera, Gracillariidae). 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. Likewise Kehrli et al (2005) found that the parasitoids of the horse chesnut leafminer are removed along with the pest during the removal of leaves from the soil as part of the sanitation. Two ingenious solutions to

overcome this problem are screened-enclosures (Kehrli et al., 2005; Klungness et al., 2005), in which all infested fruits and leaves collected from the field are placed. The screen material used for its construction prevents the dispersion of tephritid flies and chesnut moths emerging from infested fruits and leaves, but allows the escape of parasitoid wasps, thus minimizing the negative effects of crop sanitation on natural enemies (Jang et al., 2007; Kehrli et al., 2005). The use of these two devices been shown to be highly efficient in reducing fruit fly populations in the field (Klungness et al., 2005; Kehrli 2004).

Damon and Valle (2002) have reported that the efficacy of the parasitoid *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.

CHAPTER 5

Aphanogmus sp. (Hymenoptera: Ceraphronidae): a hyperparasitoid of the coffee berry borer parasitoid *Prorops nasuta* (Hymenoptera: Bethylidae) in Kenya

Abstract

This is the first report of a hyperparasitod of the primary parasitoid of the coffee berry borer *Prorops nasuta* Waterston (Hymenoptera: Bethylidae). *Aphanogmus* sp is a gregarious ectoparasitoid of larval and pupal stages of *P. nasuta*, which was found in coffee berry samples collected on the ground of an organic coffee plantation in Western Kenya. The hyperparasitoid shows a clear pattern of emergence from year to year, following its host. *Aphanogmus* sp parasitizes around 10% of *P. nasuta* immature stages under field conditions.

Keywords: Hyperparasitoid; Primary Parasitoid; Coffee Berry Borer; *Prorops nasuta*; *Aphanogmus* sp; Africa.

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The coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae) is the most important coffee pest worldwide (Damon 2000; Jaramillo et al., 2006). The insect causes serious economic losses to commercial coffee plantations, and these losses reduce earnings for more than 20 million rural families around the world (Vega et al., 2003). The females bore galleries into the endosperm of the coffee berries causing its premature fall and qualitative and quantitative losses in coffee through feeding of the larvae inside the berries (Le Pelley 1968). *Prorops nasuta* Waterston (Hymenoptera: Bethylidae), first recorded in Uganda in 1923 (Hempel 1934), is an ectoparasitoid of CBB larvae and pupae. In addition it attacks the female beetles and preys on their eggs and young larval stages (Hargreaves 1935). The parasitoid has been recorded from coffee plantations in West, Central and East Africa (Abraham et al., 1990), and has been also introduced to the Americas and to several Asian countries for biological control of CBB (Barrera et al., 1990; Baker 1999). In Uganda *P. nasuta* numbers in coffee plantations start to increase from April onwards, with peak numbers recorded during June and July, resulting in an effective control of CBB populations (Hargreaves 1926).

Here, we report on the findings of a new species of *Aphanogmus* (Hymenoptera: Ceraphronidae) as a hyperparasitoid of *P. nasuta* in Kenya. The species description will be published elsewhere.

Starting from October 2006, a 2,000-tree organic coffee plantation (*Coffea arabica* var. Ruiru 11) in the Kisii District of Western Kenya was sampled on a bi-weekly basis during 2006 and 2007, and weekly during 2008 for natural enemies of the coffee berry borer by collecting beetle-infested berries that had fallen to the ground. Thereafter, the berries were taken to the laboratory and placed in square

plastic containers (40 x 40 x 20 cm) with perforated lids (5.5 cm diameter) covered with mesh. The containers were layered with a 1.5 cm mixture of plaster of Paris and activated charcoal to retain the humidity and delay the desiccation of the berries, thereby increasing the survivorship of natural enemies within the berries (Jaramillo et al., 2008). This methodology allowed assessing the emergence of parasitoids from the coffee samples for periods of up to 90 days. So far, 1,342 individuals of *Aphanogmus* sp have been recovered since October 2006. *Aphanogmus* sp emergences commenced in both 2007 and 2008 during January and the population peaks around April. No emergence of the hyperparasitoid was recorded from July to December 2007, coinciding with very low prevalence of the *P. nasuta* in the field.

During the dissection of berries we observed that *Aphanogmus* sp spends most of its time inside the coffee berries within the coffee berry borer galleries. Before parasitizing *P. nasuta*, the hyperparasitoid probes with its antennae the older host larvae or pupae just before construction of the cocoons. Around 10% of the total number of *P. nasuta* cocoons were parasitized by *Aphanogmus* sp. The hyperparasitoid usually oviposits on the abdomen of *P. nasuta*, and up to three *Aphanogmus* sp larvae or pupae were found inside the *P. nasuta* cocoons, most often two females and one male, or only two females (Figure 2a,b). The mean size of the young *Aphanogmus* sp larvae ranges from 0.33 to 0.50 mm, and the older larvae is approximately 0.70 mm long.

This is the first report of a hyperparasitoid of a primary parasitoid of the coffee berry borer in the area of origin of the pest. Studies on the biology and ecology of the hyperparasitoid are ongoing to assess its impact on *P. nasuta* populations in the field.

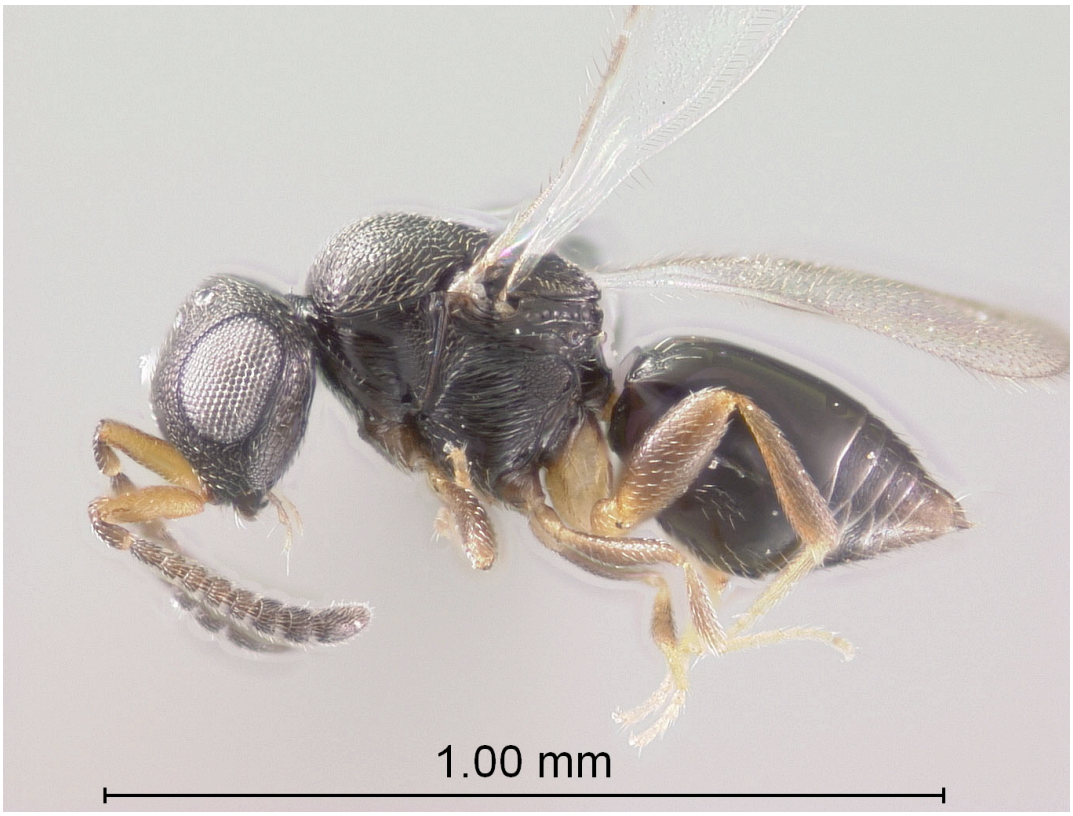


Figure 1. *Aphanogmus* sp adult. (Photo: M. Buffington and A. Simpkins, USDA)

A

B

Figure 2. *P. nasuta* pupae hyperparasitized by *Aphanogmus* sp (A). From left to right larva, pupae and adult of *Aphanogmus* sp.

CHAPTER 6

Molecular elucidation of the role of predatory thrips for biological control of the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae)

Abstract

A new predator of the coffee berry borer (CBB) *Hyopthenemus hampei* was found in the coffee growing area of Kisii in Western Kenya. Field observations, laboratory trials and gut content analysis using molecular tools have confirmed the role of the predatory thrips *Karnyothrips flavipes* Jones (Phlaeothripidae) as a specific predator of CBB.

Keywords: *Hyopthenemus hampei*; Coffee Berry Borer; Coffee; Biological Control; Predator; Gut Content analysis.

*This work is the product of a multidisciplinary and collaborative effort between Juliana Jaramillo from Leibniz Universität Hannover and the International Centre of Insect Physiology and Ecology (*icipe*), Eric Chapman and James Harwood from the Department of Entomology of University of Kentucky, and Fernando E. Vega from the Sustainable Perennial Cops Laboratory, United States Department of Agriculture (USDA), Beltsville. The results presented in this chapter are preliminary and partial and the full paper will be submitted at a later stage as: Jaramillo, J., Chapman, E.G., Vega, F.E., and Harwood, J.

Introduction

For close to 100 years coffee entomologists have been looking for a predator of the coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae), the most serious pest of commercial coffee throughout the world (Damon, 2000; Jaramillo et al., 2006). So far, the only known predators of CBB are ants, and until recently no quantitative data on the impact of this group of predators on the pest was available. A recent study by Armbrecht and Gallego (2007) reported *Solenopsis* cf. *picea* (Hymenoptera: Formicidae) as a predator of CBB under field and laboratory conditions, and concluded that these generalist predators can be important as natural enemies in shaded coffee plantations during the wet season in Colombia. However, these conclusions are based on laboratory results and some limited field observations, and no gut content analysis was used in this study. In Africa, the centre of origin of coffee and CBB, no predator has been found.

In April 2008, during routine dissections of coffee berries as part of a study that aimed at finding new natural enemies of CBB (Chapter 4) a predatory thrips feeding on immature stages was discovered.

There are 5,500 described species of thrips (Lewis, 1997). Of these, less than 50 are known to be predatory (Ananthakrishnan, 1979). Predatory thrips usually feed on small, soft-bodied insects including other thrips, aphids, scales, mites and rarely on Lepidoptera eggs (Ananthakrishnan, 1979; Lewis, 1973). The order Thysanoptera is divided in two suborders, Tubulifera and Terebrantia, and predatory thrips can be found in both of them. Terebrantia species are distributed

across seven families (Lewis, 1997) and include some effective predators belonging to the genera *Aeolothrips* Haliday, *Franklinothrips* Back, and *Scolothrips* Hinds. Of these, the most important biological control agents are *F. vespiformis* (Crawford) a predator of thrips, whiteflies, leafminers and mites under greenhouse conditions in Europe and Israel (Arakaki and Okajima, 1998; Loomans and Vierbergen, 1999), *F. orizabensis* Johansen, a predator of phytophagous thrips in avocado orchards (Hoddle et al., 2004), and *S. takahashii* Priesner a predator of spider mites (Priesner, 1950; Ding Xu et al., 2007).

On the other hand, Tubulifera species are placed in a single large family, the Phlaeothripidae (Priesner, 1964; Morse and Hoddle, 2006). In this suborder, very few species have been recorded as biological control agents. For instance *Haplothrips brevitubus* (Karny) was recently discovered in Japan preying on *Frankliniella* spp. (Thysanoptera: Thripidae) (Kakimoto et al., 2006).

Karnyothrips flavipes Jones (Phlaeothripidae) is among the oldest known predatory thrips species (Priesner, 1960). It has a wide distribution embracing North, Central and South America (Mound and Marullo, 1996), the Pacific region (Zimmermann, 1948), India (Ananthakrishnan, 1979; Pitkin, 1976), the Mediterranean, Palestine, Egypt, Europe, and South and Central Africa (Priesner 1960, 1964). *Karnyothrips flavipes* is a generalist predator that feeds mainly on scales, mites, whiteflies and other thrips, and is frequently associated with bamboo and other Graminea species (Priesner, 1960, 1964). It can also be found in fruit orchards preying on scales, mites and other herbivorous thrips in the canopy (Collins and Whitcomb, 1975; Hoddle et al., 2002; Childers and Nakahara, 2006).

Generalist predators can be effective biological control agents capable of reducing pest numbers significantly and in some cases prevent crop damage (Symondson et al., 2002). Yet, prey analysis in small-bodied generalist predator like thrips is rather challenging. Previously this was mainly done via dissection of the guts and visual examination of prey remains with a microscope (Symondson, 2002). However, this provides information only when a predator consumes an entire prey where remains like head capsules, legs etc. can be observed. However, this approach is not feasible for fluid feeders like predatory thrips. To overcome this problem a range of techniques like enzyme electrophoresis, immunological approaches using polyclonal and monoclonal antibodies, and polymerase chain reaction (PCR)-based methods have been developed during the last years to identify prey remains and elucidate the linkages between (generalist) predators and their prey (Symondson 2002; Harper et al., 2006; Harwood et al., 2007).

Here we studied the potential role of *K. flavipes* as a predator of CBB using DNA-based gut content analysis coupled with field data of the predator and the possible prey.

Materials and Methods

Field collections

Coffee berries that had fallen to the ground were sampled weekly in an organic non-shaded *Coffea arabica* L. (var. Ruiru 11) plantation (ca. 2000 trees) in the Kisii area of Western Kenya (00° 25' S, 34° 28' E, 1,510 meters above sea level [masl]). Between 100-150 trees were sampled randomly at each evaluation date,

collecting as many CBB-infested berries as possible. The berries were subsequently transferred to the laboratory, surface sterilized and placed in containers layered with a mixture of plaster of Paris and activated charcoal at room temperature (for details on the methodology see Jaramillo et al., 2008). A thrips proof net (64 μm mesh nylon net) was used to cover the perforated lids of the containers that held the coffee berries.

The thrips that emerged from the coffee berries were transferred to 0.5 ml Eppendorf® tubes containing 95% ethanol. Specimens were sent for identification to Dr. Steve Nakahara at the Systematic Entomology Laboratory of the United States Department of Agriculture, in Beltsville, USA. Throughout the study the number of emerging thrips were recorded daily.

Gut content analysis: DNA extraction and PCR protocols

Karnyothrips flavipes specimens that had emerged in the morning from field collected coffee berries that had fallen to the ground were initially starved for 36 hours and then placed in pure ethanol (99%).

Using the CBB-specific primers the PCR assay was screened for cross-reactivity with a range of other insects that are usually found inside coffee berries, to elucidate whether *K. flavipes* were also feeding on these 'non-target' species in this well-defined niche (Table 1) and to be sure that the CBB-specific primers would not give a false positive.

Total DNA was extracted from whole insect specimens, since the size of the insect is less than 1 mm which makes difficult and time comsuming the dissection of guts, using QIAGEN DNeasy Tissue Kits (QIAGEN Inc., Chatsworth, CA) following

the animal tissue protocol with one exception: after incubating at 56°C for one hour in buffer ATL of the kit and proteinase k, the insects were broken into pieces in the buffer solution with sterile pipette tips and returned to the incubator to soak overnight. Polymerase chain reaction (PCR) was performed to amplify cytochrome oxidase I (COI) from all of the insect species that have been found associated with CBB within coffee berries and CBB itself (Table 1) using the primers LCO-1490 and HCO-2198 (Folmer et al., 1994). The Folmer primers are general metazoan COI primers designed to amplify COI from a wide range of taxa. The aim was to generate sequences for all of the insects associated with the coffee berry (Table 1), in order to line them all up and identify regions where CBB had unique sequence, to target those regions for CBB-specific primers.

Table 1. Insect species found associated with the coffee berry borer within a coffee berry.

Order	**Family**	**Species**
Coleoptera	Curculionidae	*Hypothenemus hampei* (Ferrari)
Thysanoptera	Plaeothripidae	*Karnyothrips flavipes* (Jones)
Hymenoptera	Bethylidae	*Prorops nasuta* Waterson
Hymenoptera	Ceraphronidae	*Aphanogmus* sp.
Hymenoptera	Formicidae	*Tapinoma* sp.
Diptera	Tephritidae	Nn
Homoptera	Aleyrodidae	Nn

PCR reactions (total volume = 50 μL) consisted of 1X QIAGEN PCR buffer (1.5 μM $MgCl_2$), 0.2 μM each dNTP, 0.5 μM each primer, 1U QIAGEN *Taq* and an

unquantified amount of template DNA (5µl of total DNA). Additional $MgCl_2$ (final PCR concentration = 5 µM) significantly improved the COI PCR product for all species in Table 1 except for *H. hampei*, which required no additional $MgCl_2$. PCR reactions were carried out in a Bio-Rad PTC-200 thermal cycler (Bio-Rad Laboratories, Hercules, USA). The PCR cycling protocols were 94°C for 1 min followed by 50 cycles of 94°C for 45 s, 40°C for 45 s, 72°C for 45 s and a final extension of 72°C for 10 min. Electrophoresis of 10 µL of PCR product in 1.5% SeaKem agarose (Lonza, Rockland, USA) were run at 140 volts for 20-30 minutes. Afterwards gels were stained with ethidium bromide (EtBr, e.g. 0.1 mg/µL) to viualize bands on an UV transilluminator.

PCR reactions that yielded significant product were purified with QIAGEN MinElute PCR purification kit. Cycle sequencing was carried out in both the forward and reverse directions using the ABI Big-Dye Terminator mix (v. 3.0) in an ABI 9700 thermal cycler, and run out in an ABI 3730xl sequencer (Applied Biosystems, Foster City, USA). Forward and reverse COI sequences from the same individual were assembled using AlignIR (v. 2.0, LI-COR Biosciences Inc., Lincoln, USA) BLASTN (Karlin and Altschul, 1990, 1993). Searches of the GenBank database were performed on the resulting sequences to determine whether the sequences significantly matched those of the same or related species, to ensure that the sequences were not from a parasite or other contaminant. Multiple sequence alignments were done using CLUSTAL_X (Larkin et al., 2007). This alignment was used to design two pairs of species-specific COI primers (Table 2) for *K. flavipes* and *H. hampei*.

Table 2. Species-specific primers.

Species	Primer name	Primer sequence (5'-3')	Amplicon size
K. flavipes	Karyothrips_COI-25-F	CTGATCAGGAATCTGTGGCTTA	604 bp
	Karyothrips_COI-628-R	GTAGGGTCACCTCCTCCTGT	
H. hampei	CBB_COI-373-F	TTGACAAAGGAGCAGGAACA	145 bp
	CBB_COI-517-R	TTCTGGCTGTATCCCAGGAG	

One pair (Karyothrips_COI-25-F and Karyothrips_COI-628-R) was designed to amplify a 604 bp fragment of *K. flavipes* COI to check whether the DNA extractions worked (this primer pair was not screened for cross-species reactivity). This was necessary because the Folmer (1994) primers listed above were inconsistent in amplifying *K. flavipes* COI, even with the addition of $MgCl_2$ as described above. A second pair (CBB_COI-373-F and CBB_COI-517-R) was designed to amplify a 145 bp fragment of the CBB COI, and was screened for cross-reactivity against all other insects found associated with CBB within the coffee berries (Table 1). This primer was used to detect the presence of CBB DNA in *K. flavipes* DNA extractions. The PCR cycling protocol for these primers are the same as those listed above except that the annealing temperature was 56°C. To determine reaction success for PCRs utilizing the CBB-specific primers, electrophoresis of 10 μL of PCR product in 3% SeaKem agarose was done to separate the <200 bp PCR product from the glycerol-bromphenol blue-based loading dye. Positive controls containing CBB DNA and negative controls with distilled water were included in each PCR to check that the PCR was assembled properly and that there was no contamination. In total, 32 PCR reactions from a single mix tube that contained all of the PCR reaction components

except the DNA from each specimen were set up. Out of these 32 reactions, 30 contained 5μL of total DNA from 30 different thrips extractions, one contained 2μL of total DNA from a CBB extraction (the positive control), and one received PCR-grade water (the negative control). The positive control was necessary to show that the correct reaction conditions were present to amplify DNA. If a band appeared in the negative control that meant contamination, and thus a repetition of the trial was carried out.

Statistical analysis

Karnyothrips flavipes emergence data were analyzed, considering months (April to July 2008) as a repeated parameter, by means of repeated measures two-ways ANOVA, using the general linear model (PROC GLM) of SAS (1999) to determine single or interaction effects of factors. In case the ANOVA yielded significant *F*-values, emergence means were compared using Tukey's test (HSD). To identify particular time intervals in which emergence of *K. flavipes* is different, individual ANOVAs (*F*-tests) were computed. The significance level was set at $P = 0.05$.

Results and Discussion

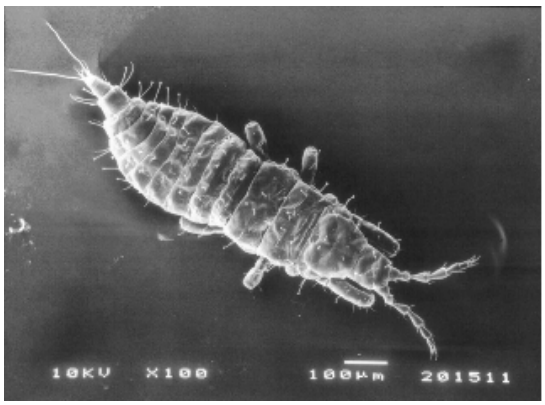
Identification of specimens

The specimens were slide mounted and identified as *Karnyothrips flavipes* (Jones) (Phlaeothripidae) by Dr. Steven Nakahara of USDA Beltsville (USA). The genus has at least 11 synonyms and has been treated in 10 genera. It is a predatory thrips. According to Priesner (1960) (treated as *Watsoniella flavidus*), the larvae and

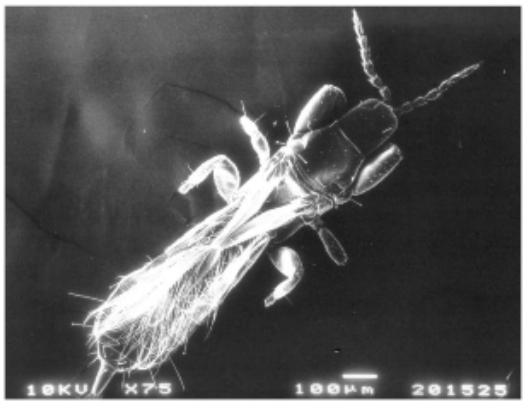
adults feed on the eggs and larvae of several species of soft scales (Coccidae), and armored scales (Diaspididae), as well as on mites and whiteflies. This is the first time *K. flavipes* has been reported being predacious on different Coleopteran life stages and associated with non-Gramineous plants (S. Nakahara., pers. comm.).

Observations in the laboratory

In April 2008, during routine dissections of coffee berries, *K. flavipes* (Figure 1) adults were observed feeding on eggs of *H. hampei* inside the galleries constructed by the female borer (Figure 2). Subsequent assays in the laboratory revealed that *K. flavipes* is also able to prey on larval stages of CBB (Figure 3). This constitutes the first report of *K. flavipes* being associated with coffee and preying on different immature life stages of a Coleopteran species. Likewise, this is the first time a predator of CBB has been found in Africa.



a



b

Figure 1. *K. flavipes* (a) larvae, and (b) adults. (Photos: M. Chimtawi, *icipe*)

Figure 2. Adults of *K. flavipes* preying on eggs of the coffee berry borer (CBB) inside CBB galleries.

Figure 3. *Karnyothrips flavipes* adults feeding on eggs and larvae of the coffee berry borer.

The thrips oviposit inside the coffee berries between the pulp and the parchment of the beans, and up to 29 eggs in a single berry were found during the dissections (Figure 4). *Karnyothrips flavipes* adults spend most of their life inside the

CBB galleries, whereas the thrips larvae usually were often found between the two coffee seeds. When starved the thrips do not survive longer than 48 hours.

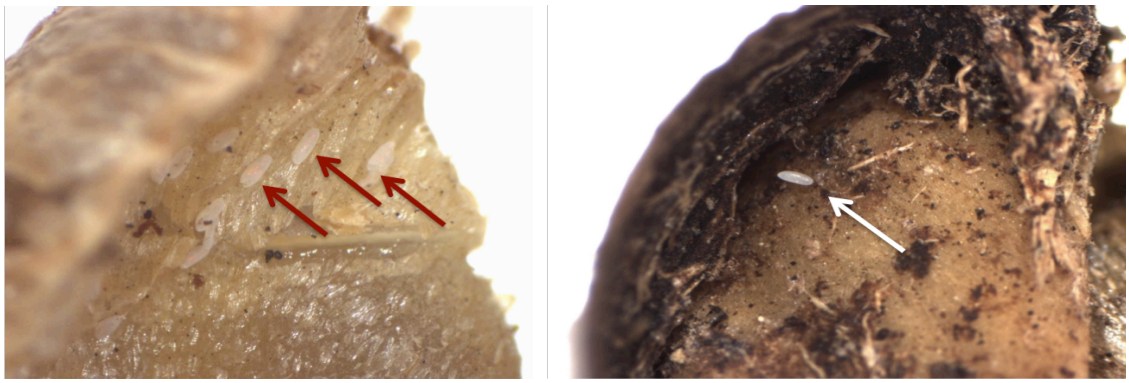


Figure 4. *Karnyothrips flavipes* eggs oviposited inside the coffee berries.

Abundance of Karnyothrips flavipes *from field collected coffee berries*

In total 2,990 *K. flavipes* individuals emerged from 16,546 CBB-infested berries collected from the ground between April to July 2008. Figure 3 presents the monthly emergence of thrips per 100 berries.

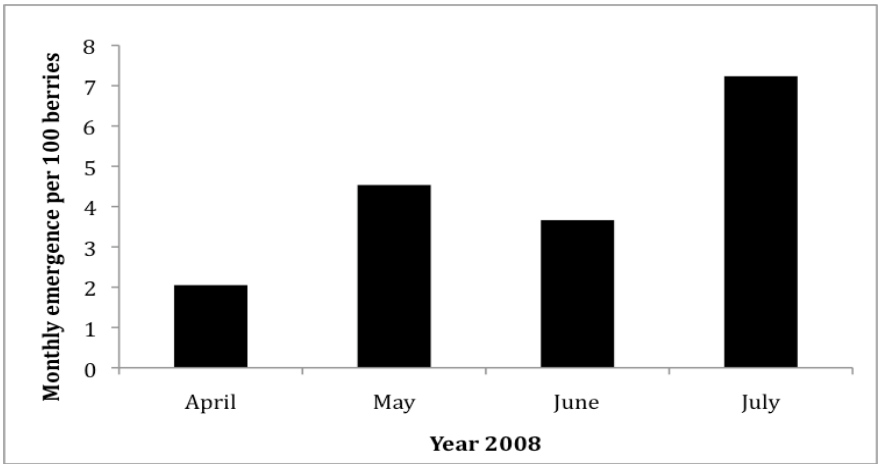


Figure 3. Monthly emergence of *Karnyothrips flavipes* per 100 CBB infested coffee berries collected from the ground.

The numbers of predatory thrips recovered over time differed significantly ($F = 14.47$, $P < 0.0001$) and in general, the emergence of *K. flavipes* increased over time ($F = 9.16$, $P < 0.0001$) (Figure 4).

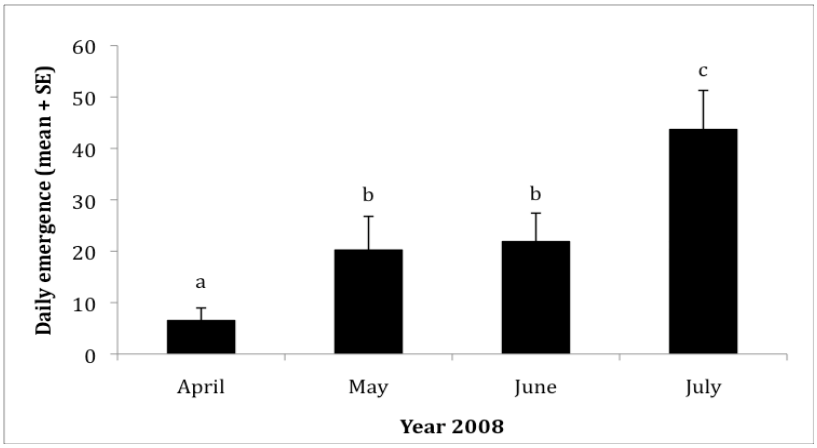


Figure 4. Daily emergence (mean ± SE) of *Karnyothrips flavipes* from coffee berries collected from the ground (n =16,546).

Gut content analysis: DNA extraction and PCR protocols

The *K. flavipes* specific primers (Table 2) were used to confirm that >95% of the DNA extractions of this species raised from berries were successful (e.g., Figure 5). In Figure 5, only the PCR reaction in lane 5 of the bottom row did not yield detectable PCR product. This was a typical result for the 509 DNA-extracted *K. flavipes* specimens that emerged from the coffee berries and that were analysed.

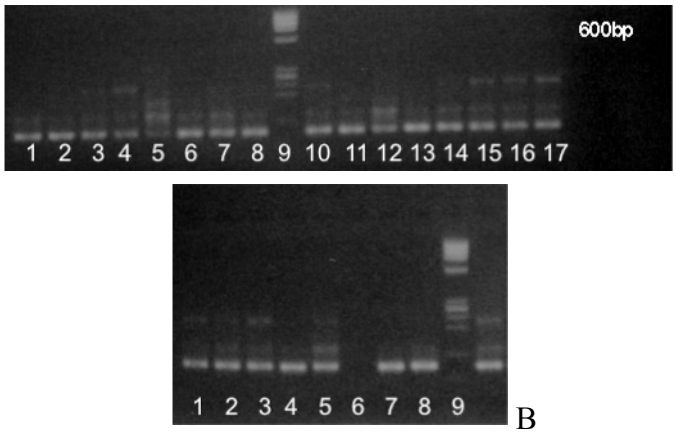


Figure 5. Agarose gel of PCR products using *Karnyothrips flavipes*-specific primers (Table 2) to determine whether the DNA extractions were successful. A) Lanes 1-8 and 10-17: successful amplification of a ~600 bp COI amplicon from extractions of *K. flavipes* specimens that emerged from coffee berries; Lane 9 contains PhiX 174/Hae III marker DNA which yields the following 11 discrete fragments (in bp): 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72. B): Lanes 1-6: *K. flavipes*; Lanes 7-8: negative controls; Lane 9: PhiX 174/Hae III marker DNA.

The CBB-specific primers (Table 2) did not produce PCR products for any of the other insects commonly found inside coffee berries (Table 1) except for *H. hampei* itself (Table 1), indicating that the CBB specific primers are indeed specific. Figure 6 shows an agarose gel loaded with PCR reactions containing the following: Lane 1: PhiX 174/Hae III marker DNA; Lanes 2-4: Coffee berry borer PCR amplicon (positive result); Lanes 5-6: starved *K. flavipes*; Lanes 7-8: *Prorops nasuta*; Lane 9: negative control. These primers did not produce PCR product for any of the other species listed in Table 1.

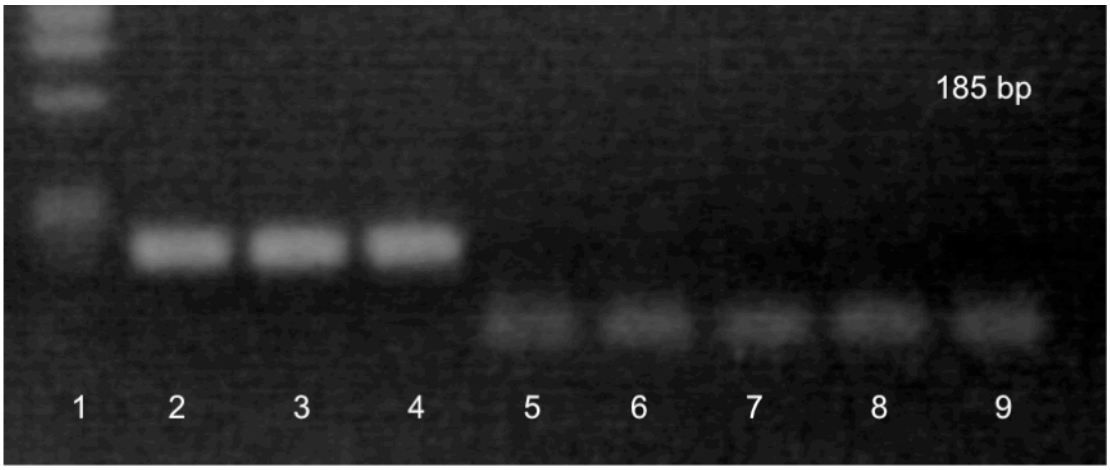


Figure 6. Agarose gel of PCR products using CBB-specific primers (Table 2) to determine if these primers will produce a product from other species commonly found inside coffee berries. The amplified PCR product length is 185 bp (e.g., 145 bp plus an additional 40 bp coming from the primer). Lane 1: PhiX 174/Hae III marker DNA; Lanes 2-4: CBB amplicon (positive result); Lanes 5-6: starved *K. flavipes*; Lanes 7-8: *Prorops nasuta*; Lane 9: negative control.

Detection of coffee berry borer DNA in Karnyothrips *DNA extractions*

CBB DNA was detectable in the DNA extractions of *K. flavipes* (96/509 = 18.9%). Figure 7 shows an example of an agarose gel containing products from PCR amplification of CBB's COI DNA from DNA extractions of *K. flavipes* specimens that were collected upon emergence from a coffee berry. In this figure, a positive result can be observed in lanes 3, 4, 10, 15-17 of the top row, and lanes 1-3, 5, and 10-14 of the bottom row (lanes 16, 17 and 19 are positive controls; nothing was loaded in lane 18).

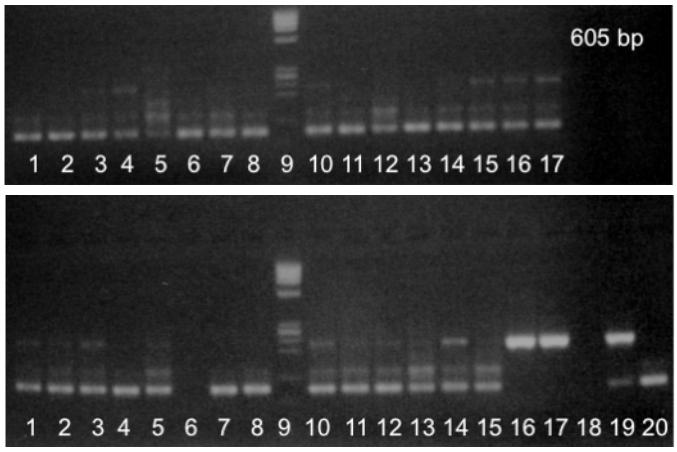


Figure 7. Agarose gel of PCR products using CBB-specific primers (Table 2). A) Lanes 1-8 and 10-17: *K. flavipes* specimens that emerged from a coffee berry; Lane 9: PhiX 174/Hae III marker DNA. B) Lanes 1-8 and 10-15: *K. flavipes* specimens that emerged from a coffee berry; Lanes 16, 17, 19: CBB DNA (positive controls); Lane 9: PhiX 174/Hae III marker DNA; Lane 20: negative control. Nothing was loaded in lane18.

In summary these results confirm that *K. flavipes* is a frequently occurring predator of immature life stages of CBB in Western Kenya. This is the first report ever of a predator of *H. hampei* in Africa. In ongoing studies the impact of this new natural enemy of CBB and its potential use for biological control are being quantified.

CHAPTER 7

Development of a new laboratory production technique for Coffee Berry Borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae), using fresh coffee berries*

Abstract

The suitability of a mixture of plaster of Paris and charcoal as a means to regulate the moisture content of coffee berries and the relative humidity (moisture conditions) of the rearing environment and its impact on rearing the coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) was evaluated under laboratory conditions using two types of coffee. Coffee berries were individually kept in vials filled with a 1 cm layer of the mixture, and the fresh weight of the berries as well as the penetration of CBB into the berries, its survival and its progeny production were assessed over a period of 55 days. Significantly higher survival and progeny production was achieved when using the mixture of plaster of Paris regardless of the coffee type. Compared to the control a 6-7-fold increase in survivorship of the F1 was recorded when using plaster of Paris, and in the latter treatment berries harboured on average more than 100 individuals vis-à-vis 1.7 in the control.

Keywords: Coffee berries; *Hypothenemus hampei*; Rearing; Borer; Total Progeny; Wet Weight.

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Introduction

The Coffee Berry Borer (CBB) *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae) is the most important coffee pest worldwide (Le Pelley, 1968; Jaramillo et al., 2006). *Hypothenemus hampei* females bore galleries into the endosperm of the coffee berries causing qualitative and quantitative losses in coffee through feeding of the larvae inside the berries (Damon, 2000). Present control strategies largely rely on applications of broad-spectrum synthetic insecticides (e.g., Mejia and Lopez, 2002) but growing environmental concerns and increasing problems with insecticide resistance in *H. hampei* (Brun et al., 1994; Gongora et al., 2001) have stimulated the search for environmentally more friendly control strategies against the pest (Jaramillo et al., 2006). Moreover, sustainable coffee production and certification schemes stress the safety aspects of pest control such, which leads to an increased demand for biological control solutions (Jaramillo et al., 2006). Prerequisites for successful inundative biological control are sound knowledge of the pest's biology, and, in the case of *H. hampei*, rearing of large numbers of healthy females at low costs for the production of natural enemies.

In spite of the economic importance of the pest, there are still major gaps in our understanding of the biology of CBB. For instance conflicting data on important life table parameters are reported in the literature (i.e., Bergamin, 1943; Ticheler, 1963; Decazy, 1990; Barrera 1994; Montoya and Cardenas, 1994; Ruiz, 1996; Fernandez and Cordero, 2007). Such differences are most likely due to the difficulties of studying a concealed pest like CBB under controlled conditions and suggest problems with the existing methodologies (Damon, 2000). Because of the difficulties

to maintain CBB on fresh coffee berries in the laboratory, some of the previously cited studies have been conducted under field conditions. Yet varying environmental factors lead to variations in reported biological parameters of the insect (e.g. Ruiz, 1996; Fernandez and Cordero, 2007).

Most of the previous efforts in mass rearing of CBB were aimed at the development of suitable production systems for natural enemies, especially parasitoids, of the pest. To date several highly efficient techniques exist, like artificial diets for *H. hampei* (Brun et al., 1993, Villacorta, 1985, Portilla 1999), a rearing methodology developed by Cenicafe, the national coffee research institute of Colombia, that uses parchment coffee (Benavides and Portilla, 1990), and a rearing methodology for the eulophid adult endoparasitoid of CBB *Phymastichus coffea* LaSalle that uses fresh coffee (Infante et al., 1994). However, for basic studies of the biology of the beetle all these techniques have considerable drawbacks. For instance, the existing artificial diets of CBB negatively affect fecundity and sex ratio of the beetles (Portilla and Streett, 2006). The method developed by Infante et al. (1994) does not permit the F1 of the colonizing female to complete its life cycle because of the desiccation of the berries. Finally, parchment coffee is comparatively expensive and not the natural substrate of the beetle. Thus, a new and affordable methodology for CBB mass rearing that uses fresh coffee berries, the natural host of the pest, thereby mimicking field conditions in the laboratory, is needed to study the beetle's biology under controlled conditions. We here report such a technique that enables efficient production of CBB in the laboratory on fresh coffee berries.

Materials and Methods

General procedure

The study was carried out in the laboratories of the International Centre of Insect Physiology and Ecology (*icipe*) in Nairobi, Kenya. For the study, coffee berries were collected from two locations in two coffee growing regions of Kenya, i.e. the Kisii district of Western Kenya (Latitude 00° 25' S, Longitude 34° 28' E, 1,720 meters above sea level [masl]) and the Kiambu district of Central Kenya (Latitude 1° 10' S, Longitude 36° 49' 60 E, 1,723 masl). In the two plantations, coffee (*Coffea arabica* var. Ruiru 11) was produced organically without use of any pesticides. The two coffee plantations were chosen based on the differences in the management of the crop as well as differences in environmental conditions, mainly water deficiency/availability. The coffee from Kisii was produced by poor small-scale farmers without any fertilizer input. This coffee plantation, in addition suffered from water deficiency during the fruiting period, resulting in a 'low quality' coffee. On the other hand, the coffee from Kiambu is cultivated using high standards of organic production, was fertilized with compost and manure and did not suffer from water stress making it a 'high quality' coffee. Thus, the present study aimed at testing whether the new rearing technique would be affected by coffee berry quality. Coffee berries used in all experiments were collected in Kisii and Kiambu, between October 2006 and January 2008. Berries older than 120 days of development time contain more than 20% of dry matter content and are therefore suitable for the development of the coffee berry borer (Alonzo, 1984; Ruiz, 1996). Coffee berries of 150 days (n = 1,500) were randomly sampled in the field. Coffee berries were collected, brought into the laboratory and checked for

infestation. Only non-infested berries of uniform shape and weight were used for the experiments. A total of 1200 berries were used for the experiments.

Hypothenemus hampei females used in this study were obtained from a colony maintained at the *icipe* laboratories. The colony was established in July 2005 with CBB infested coffee berries collected from different plantations in Kisii. The field-collected beetles were reared in plastic jars filled with three centimetres of plaster of Paris on fresh coffee berries of approximately 150 days of development collected in the Kisii area. Every month, new insects were brought to the colony. All experiments were conducted at 25 ± 1°C and 70 ± 5 % relative humidity (rh) and L12:D12 photoperiod.

Artificial infestation of the coffee berries with H. hampei females

Non-infested healthy coffee berries approximately 150 days old were selected for the experiments. Initially berries were surface sterilized using the protocol developed by Perez et al. (2005) where the berries were washed with detergent for 15 minutes, rinsed with tap water, then dipped in a 2% sodium hypochlorite solution for 10 minutes, rinsed again with sterile distilled water, thereafter soaked in a 2% potassium sorbate solution and finally rinsed with sterile distilled water. Subsequently the coffee berries were allowed to dry at room temperature (25 ± 1°C). Thereafter, the berries were placed in a round plastic container (23 cm diameter × 6.8 cm depth) and exposed to large numbers of *H. hampei* females from the stock culture. After two hours of exposure, berries that were attacked by one female per berry were selected and transferred individually into the vials (Figure 1) (see below).

Rearing procedure

The rearing containers used in this experiment are polystyrene conical shaped vials, 5 cm height, 3 cm diameter at the top and a square bottom of 2x2 cm. The containers had plastic lids with an opening of 1.5 cm diameter, which was covered with insect gauze to prevent the escape of CBB females. Depending on the treatment, the vials were either filled or not with a one cm layer of a mixture of plaster of Paris and charcoal (9:1) as used by Premachandra et al. (2005) in their study on the biology of *Ceratothripoides claratis* Shumsher (Thysanoptera: Thripidae). Infested and non-infested coffee berries were placed individually into the vials with or without the plaster of Paris mixture. In total, four treatments were evaluated as follows: (i) infested berries in vials with the plaster of Paris mixture, (ii) infested berries in vials without the plaster of Paris mixture, (iii) non-infested berries in vials with the plaster of Paris mixture, and (vi) non-infested berries in containers without plaster of Paris. All the vials were completely randomized. Six hundred vials were used for each treatment, given a total of 300 borer females reared per treatment. The whole procedure was replicated three times for the two coffee berries sources (i.e., Kisii or Kiambu). Sterile distilled water was added every three days to the vials that contained the plaster of Paris mixture to keep the moisture and prevent desiccation of the berries. The vials that did not contain the layer of plaster of Paris were not watered to prevent fungal or bacterial contamination arising from completely soaked coffee berries. In addition, the emergence of CBB females is triggered when the infested coffee berries are soaked in water (Baker, 1999). The vials were kept at room temperature (25 ± 1°C and 70% ± 5% rh and L12:D12 photoperiod) to mimic normal rearing conditions.

Evaluation of treatments

According to Bustillo et al. (1998), the positions of the CBB female in the coffee berry are defined as: position A, when the female is starting to colonize a new berry and the penetration of the exocarp begins; position B, when CBB has commenced penetrating the berry but has not yet reached the endosperm; position C, when the beetle has started to bore into the endosperm but not to oviposit; 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. For each position A, B, C and D, numbers of alive and dead CBB females as well as the number of CBB life stages (i.e., eggs, larvae an adults) were recorded at 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55 days after infestation. At each evaluation date a sample of five berries per treatment and coffee source (i.e., Kisii or Kiambu Districts) was taken. After recording the position of the colonizing female inside the berry, the coffee berry was dissected under a stereomicroscope (10X), and the number of CBB eggs, larvae and females were counted.

For the non-infested berries, their wet weight was recorded at each evaluation date.

Statistical analyses

The percentage of colonizing females in each position (A, B, C and D) was calculated and mortality/ survival of colonizing females as well as that of life stage estimated across evaluation dates. The differences in mortality/survival between treatments and sources of berries (i.e., Kisii and Kiambu Districts) were analyzed using a χ^2 test.

Differences in oviposition period, total number of eggs, total progeny, sex ratio, and egg-adult survival were analyzed by analysis of variance (ANOVA), using the general linear model (GLM) procedure of SAS (SAS, 1999). A Bonferroni test was used to test the significance of mean differences between treatments.

The change in the coffee berries' wet weight over time was described by fitting the data to a modified equation of Sequeira and Mackauer (1992) using a non-linear least square regression:

$$H = (1/a1)*[1+ \exp (a_2-a_3 *t)]$$

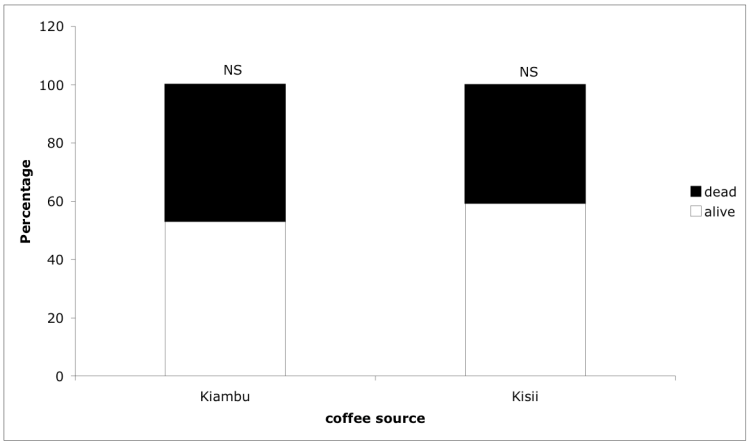
Where, H is the weight of the coffee berry in grams (g), t is days after infestation with CBB, and a_1, a_2 and a_3 are fitted coefficients. All the fitted coefficients were estimated using the non-linear model (PROC NLR) procedure of SAS (SAS, 1999). The difference in the change of the coffee berries' wet weight between treatments over time was analyzed by analysis of variance using the area under curve method in the proc GLM (SAS, 1999) procedure for repeated measures over evaluation dates. An *F*-test was used to test the significance of mean differences and least square mean values were computed. The significance level was set at $P = 0.05$.

Results

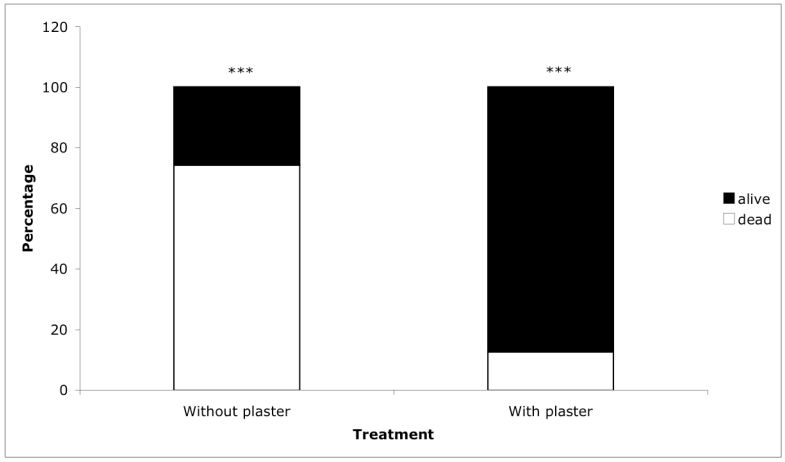
Effect of plaster of Paris on the colonization of coffee berries and mortality by/of CBB colonizing females

The proportion of alive or dead CBB colonizing females as a function of treatment are shown in Figure 1. The presence or absence of plaster of Paris in the experimental units significantly affected the mortality/ survival of the CBB females

(χ^2 = 182.19, (1, N = 472) $P < 0.0001$). The percentage of alive females found in the treatments with and without plaster was 87 and 26 %, respectively. No significant differences were found between coffee sources for any of the treatments (χ^2 =1.838, (1, N = 472), P = 0.1751). The effect of plaster of Paris in the rearing vials on the survival of CBB for the two different coffee sources is presented in Figure 2.



A



B

Figure 1. Proportion of alive and dead coffee berry borer (CBB) females attacking coffee berries from two sources, i.e. the Kisii and Kiambu Districts of Kenya (A). Proportion of alive and dead CBB females found in coffee berries kept in vials with or without plaster of Paris (B). NS and *** indicate non-significant and significant differences, respectively.

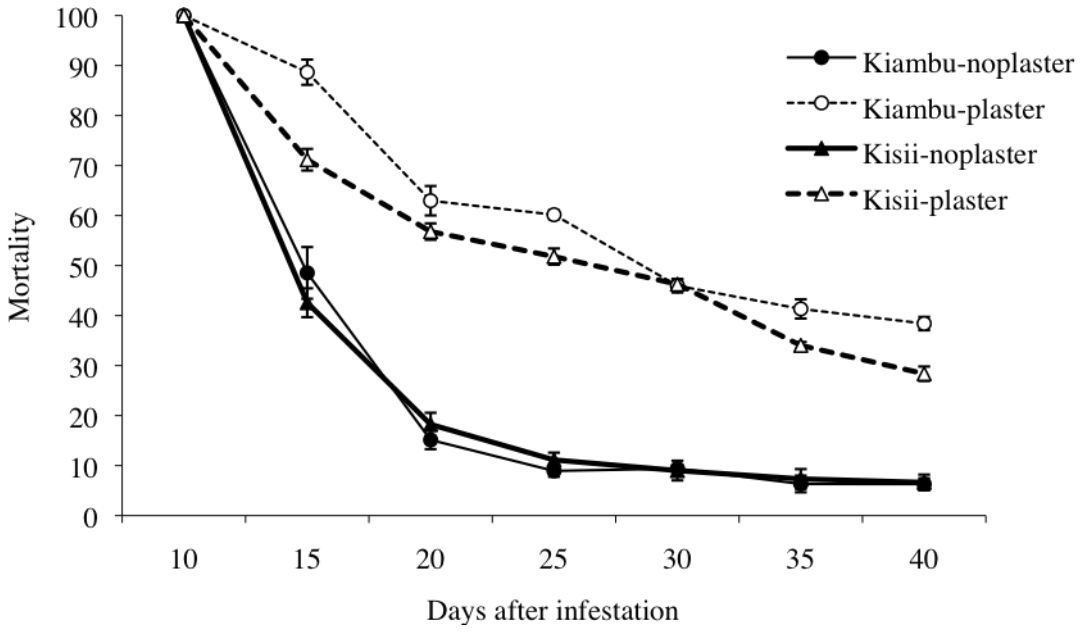


Figure 2. Effect of plaster of Paris on coffee berry borer survival in two different sources of coffee berries (i.e. Kisii and Kiambu Districts of Kenya).

No CBB female was found in position A. The percentage of colonizing CBB females that were found in positions B, C or D was significantly affected by the coffee source ($\chi^2 = 28.09$, (1, N = 602), $P < 0.0001$) and the plaster of Paris treatment ($\chi^2 = 76.73$, (1, N = 602), $P < 0.0001$) (Figure 2). In the plaster of Paris treatment the highest proportion of CBB females were found in position D (75 %), followed by position C (21 %), whereas for the non-plaster treatment, the proportions were 48 and 40 % for positions C and D, respectively.

Effect of plaster of Paris on the reproductive potential of CBB inside coffee berries

Data on the oviposition period, total number of eggs and progeny per coffee berry, sex ratio and egg-adult mortality are presented in Table 1. Dissections of the

coffee berries revealed that in the vials without plaster of Paris most of the CBB died in the larval and pupal stages.

The presence or absence of plaster of Paris in the vials for the two coffee sources (i.e., Kisii or Kiambu) had significant effects on the oviposition period (F = 90.25, P = 0.0007) (F = 361, P < 0.0001), total number of eggs per berry (F = 64.35, P = 0.0013) (F = 11.47, P = 0.0267), total progeny per berry (F = 24.12, P = 0.008) (F = 84.9, P = 0.0008) and egg-adult survival (F = 330.61, P < 0.0001) (F = 103.99, P = 0.0005).

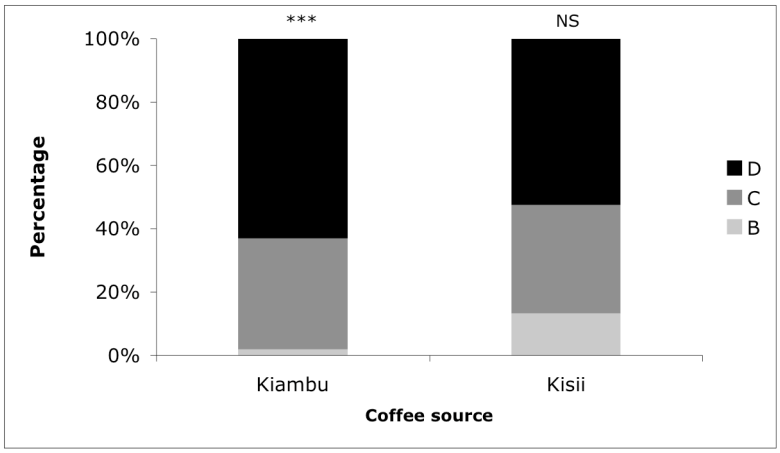
Table 1. Effect of plaster of Paris on the survival and reproduction of coffee berry borer (CBB) in two different sources of coffee berries (i.e., from the Kiambu and Kisii Districts of Kenya).

		Development and reproduction attributes of CBB				
Coffee	**Treatment**	**Oviposition period (days)**	**Total eggs per berry**	**Total progeny per berry**	**Sex ratio[1]**	**Egg-adult survival (%)**
Kiambu	No Plaster	15.0±2.9a	51.0±26.2a	1.7±0.9a	0.0±0a	6.3±1.16a
	Plaster	46.7±1.7b	287.7±13.5b	107.3±21.5b	88.0±3.3b	38.4±1.3b
Kisii	No Plaster	18.3±1.7a	44.7±15.5a	2.3±0.9a	0.0±0a	6.6±1.6a
	Plaster	50.0±0.1b	144.7±25.1b	41.7±4.2b	88.2±1.5b	28.4±1.4b

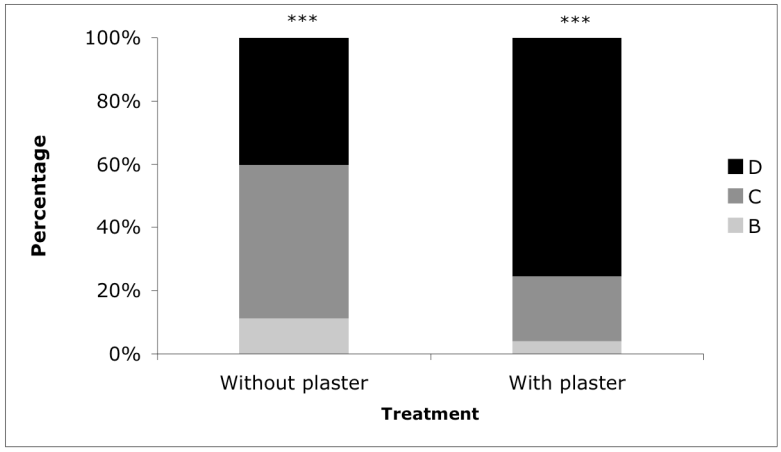
Means followed by the same small letter in each by coffee source are not significantly different (P > 0.05, Bonferoni test). Note: [1], Percentage of female in the total progeny

No F1 females were found in the berries that were reared on the non-plaster treatment for both coffee sources (Table 1). Average egg-adult survival after 50 days of infestation was 6.3 (*se* = 1.2), 6.6 (*se* = 1.6), 38.4 (*se* = 1.3) and 28.4% (*se* = 1.4)

for the treatment without plaster and with plaster, for Kiambu and Kisii, respectively (Figure 3).



A



B

Figure 3. Proportion of coffee berry borer (CBB) females found in each position of penetration into the coffee berries (B, C and D), A) in coffee berries from two different sources (i.e. the Kiambu and Kisii Districts of Kenya) and B) when the infested berries were exposed to vials with or without plaster of Paris. NS and *** indicate non-significant and significant differences, respectively.

Effect of plaster of Paris on coffee berries weight on developmental period of CBB

The coffee berry wet weight decreased significantly ($F_{(9, 72)} = 292.28$; $P < 0.001$) over evaluation time, across all treatments (Figure 4).

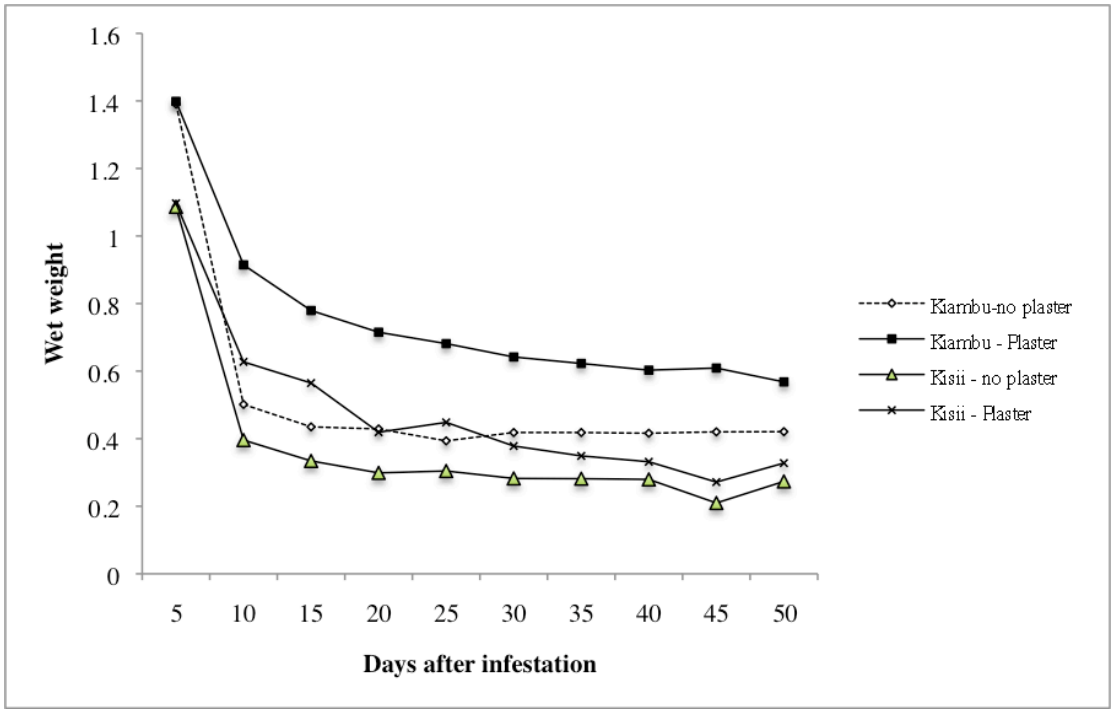


Figure 4. Effect of plaster of Paris on the change in coffee berries' wet weight (g) over the evaluation time (1/wet weight). The coffee originated from the Kiambu and Kisii Districts of Kenya.

The non-linear model gave a good fit to the data sets for both coffee sources and the two treatments ($r^2 = 0.438$, $F = 1107.58$, $P < 0.0001$), ($r^2 = 0.704$, $F = 1505.25$, $P < 0.0001$) ($r^2 = 0.283$, $F = 773.92$, $P < 0.0001$) ($r^2 = 0.804$, $F = 1206.84$, $P < 0.0001$) for coffee source Kiambu, treatments without plaster and plaster, and coffee source Kisii, treatments without plaster and plaster, respectively. The fitted parameters of the model are presented in Table 2. The change in the coffee berry wet weight over the evaluation time varied significantly ($F_{(3, 8)} = 10.31$; $P = 0.004$) between treatments. The total wet weight reduction in the coffee berries was significantly lower ($P < 0.01$) in Kiambu coffee berries reared on plaster of Paris compared to the other three treatments (Figure 4).

Table 2. Fitted parameters of the model.

Coffee source	Treatment	Parameters		
		a1	a2	a3
Kiambu	Without plaster	0.9837	-14.3648	-6.8408
	With plaster	8.8943	2.4792	-0.0150
Kisii	Without plaster	8.4093	3.0214	-0.0328
	With plaster	1.1397	-54.8397	-5.4475

Discussion

A coffee berry starts to desiccate the moment it is picked from the tree. In our study we used berries of 150 days that on average have 64% moisture content (Montoya and Cardenas, 1994). As CBB can only thrive in berries that have a minimum moisture content of 40% but not higher than 80% (Montoya and Cardenas, 1994; Bustillo et al., 1998), this assured a suitable period for the development of the beetles' progeny. Once the moisture content drops beyond this critical level, CBB mortality sharply increases (Benavides and Portilla, 1990). However, field data from Colombia suggest that berries harbouring the pest and which felt to the ground are a tremendously important source for the re-infestation of next season's coffee (Baker, 1999; Bernal et al., 1999; Bustillo et al., 1999). Bergamin (1944) and Salazar et al. (1993) observed that CBB continues to reproduce and develop in the fallen berries, and assumed that the wet soil surface slows down the desiccation process of the berries, thereby increasing the survivorship of the beetles inside. The experimental

set-up used in this study tried to mimic the phenomenon in the laboratory through a technique that can delay the desiccation of a coffee berry by utilizing a mixture of plaster of Paris and charcoal. The combination of these two materials allows to better regulate the relative humidity in the environment, thus slowing down the dehydration of the berries, and at the same time preventing them from rottening. The buffering potential of this mixture has been successfully used in cultures of insects like thrips (Premachandra et al., 2005) and Collembola (Fox et al., 2007; Park, 2007), and mites (Muma and Denmark, 1967). Relative humidity and moisture content of the berries are important factors for the appropriate development and progeny production of CBB; hence their optimal regulation are extremely critical for any rearing system (Baker et al., 1992ab; Baker et al., 1994). In our methodology, the microclimate inside the vials with plaster of Paris kept the berries for a longer time fresh, as shown by the slower decrease of weight in these berries over time compared to the control. This favoured the development of the borer as it provided sufficient time for the eggs to hatch and the larvae to develop into pupae. When finally the berries started to desiccate further, a considerable proportion of the F1 had already moulted into pupae or adults. In general older CBB broods can better withstand desiccation than younger ones (Baker et al., 1994). Compared to the control a 6-7-fold increase in survivorship of the F1 was recorded when using plaster of Paris, and in the latter treatment berries harboured on average more than 100 individuals vis-à-vis 1.7 in the control.

The coffee from Kisii was produced by poor small-scale farmers without any fertilizer input. This coffee plantation, in addition suffered from water deficiency during the fruiting period, resulting in a ‘low quality’ coffee. On the other hand, the coffee from Kiambu is cultivated using high standards of organic production, was

fertilized with compost and manure and did not suffer from water stress making it a 'high quality' coffee. Beetles in the plaster of Paris treatment were less affected by the different quality of the coffee berries.

Coffee berries in the control dehydrated very fast, thereby forcing the colonizing females to stop their penetration into the berry in positions B or C (Figure 4B). Offspring of females in position D either died of desiccation or was carried out of the berry by the colonizing females (J. Jaramillo, unpublished data). The latter behaviour was also observed by Baker et al. (1994), and they hypothesized this to be a form of brood hygiene. However, we believe that the colonizing female tries to place its brood in a more suitable, i.e. moist environment to increase its survival. Yet, no F1 adults were observed in the control.

In this study CBB progeny production was assessed for a period of 55 days. The survivorship of the F1 started to fall below 50% 40 days after infesting the berries in the plaster of Paris treatment; thus maximum survivorship of the F1 did not exceed 37%. Most likely berries started to dehydrate under our experimental conditions beyond the critical point of 40% moisture content (Bustillo et al., 1998) after 40 days. However, using multi well plates with 12 berries instead of an individual berry in a vial, progeny production can be extended until the F2 to F3, probably because of the higher relative humidity in such an experimental set-up (J. Jaramillo, unpublished data).

Although we did not quantify the microbial contamination of the berries over time, its extend was marginal and never affected the CBB brood, probably due to the efficient initial surface sterilization of the berries and because the plaster of Paris

effectively buffered the relative humidity in the experimental units. This capacity maybe particularly important for CBB production systems in the coffee growing regions of the Americas where the ambient relative humidity can reach up to 100% for several months. For example in Mexico, the use of fresh coffee berries for CBB production had to be abandoned and replaced by artificial diets (Villacorta, 1985; Villacorta and Barrera, 1993) mainly because of problems with microbial contaminations (F. Infante, personal communication).

We conclude that the here proposed methodology is cheap, easy to implement and not labour intensive. It is particularly well suited for conducting experiments under controlled laboratory conditions or to establish small CBB colonies.

CHAPTER 8

Thermal tolerance of the coffee berry borer *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae: Scolytinae): inferences of climate change impact on a tropical insect pest*

Abstract

The impact of climate change on natural processes and on biological systems is one of the most critical issues faced by mankind. Coffee, a crop on which more than 100 million people in the tropics depend for their subsistence, is predicted to be severely affected by climate change. We determined the thermal tolerance of the coffee berry borer, *Hypothenemus hampei*, the most devastating pest of coffee throughout the world, and make inferences on the possible effects of climate change on the insect using climatic data from Colombia, Kenya, Tanzania, and Ethiopia. The extremes for coffee berry borer survival are 15 and 30°C, but development takes place only between 20 and 30°C. Our thermal tolerance estimates indicate that the reason why the insect is not present in certain regions of Ethiopia is the low mean annual minimum temperatures prevalent there, and not plant resistance, natural enemies, etc. Our model suggest that a small increase in temperature will lead to faster insect development and based on the fact that *H. hampei* feeds solely on coffee, it will likely track any latitudinal and/or altitudinal movement of the plant leading to increased pest pressure and yield losses in the reduced coffee production areas. The negative effects of climate change on coffee production could be alleviated by shade trees in coffee plantations, which mitigate microclimatic extremes by decreasing temperatures up to 4°C. Such reductions in temperature, coupled with higher biodiversity in plantations could reduce pest pressure and increase yields.

Keywords: *Hypothenemus hampei*; Bionomics; Thermal Tolerance; Coffee Berry Borer; *Coffea arabica*; *Coffea canephora*; Temperature; Climate Change; Tropics.

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Introduction

The impact of climate change on natural systems has emerged as one of the most critical issues faced by humankind. According to the Intergovernmental Panel on Climate Change (IPCC, 2007), an increase in the mean global temperature of 1.4° to 5.8° C is expected by the end of the 21st century (Houghton et al., 2001). IPCC (2007) provides an overview of our scientific understanding on climate change, and this assessment offers evidence of impact on, among others, natural biological systems (IPCC, 2007). Global climate change is likely to directly influence the dynamics of all trophic levels and further disrupt the multitrophic interactions among the different communities (Parmesan and Yohe, 2003; van der Putten et al., 2004).

In addition, climate change represents an immediate and unprecedented threat to agriculture. A 10–20% decline in overall global crop yields is predicted by 2050 (IPCC, 2007). This is of particular importance for crops such as coffee, which serves as the economic foundation for many countries in the tropics, and on which millions of people depend for their subsistence. One such crop is coffee. Out of 103 species in the genus *Coffea* (Rubiaceae), only two are commercially traded: *C. arabica* L. and *C. canephora* Pierre ex A. Froehner (Davis et al., 2006; Vega et al., 2008). In terms of monetary value, coffee is the most heavily traded commodity in the world after oil (Vega, 2008). Around 70% of the world's coffee is produced by small-scale farmers, with over 20 million coffee-farming families – equivalent to more than 100 million people - depending on its production for their subsistence (Vega et al., 2003a). Recent studies from Brazil, Mexico and Uganda show that even minimal increases in the mean temperature due to climate change will have disastrous consequences for coffee

production, in some cases reducing the area presently suitable for coffee production by 95% (Grid, 2002; Assad et al., 2004; Gay et al., 2006). *Coffea canephora* (widely known as robusta coffee) is native to humid forests or the lowland forests of the Congo River Basin, an area with elevations ranging from 0-1,200 meters above sea level (m.a.s.l.) (Davis et al., 2006), and an average temperature of 24-26°C (Coste, 1993). *C. arabica*, regarded as the highest quality coffee, is native to the highlands of South Western Ethiopia where it grows naturally as an understory tree in forests at elevations ranging from 1,600 - 2,800 m.a.s.l. (Davis et al., 2006), and an average temperature of 18-21°C (Alegre, 1959). Above or below these temperatures the yield and quality of *C. arabica* is greatly reduced (Pinto et al., 2001; Assad *et al.*, 2004; Damatta and Cochicho-Ramalho, 2006). Of the total world coffee production, 60% is Arabica coffee (ICO, 2008) (Davis et al., 2006). Since the vast majority of *C. arabica* and *C. canephora* are grown in the tropics they are especially vulnerable to global climate change (Addo-Bediako et al., 2002). Climate-induced stress may render plants more vulnerable to opportunistic herbivores (Cannon, 1998). Furthermore, the direct effects of temperature on herbivores are likely to be larger and more important than any other factor associated with climate change like drought, CO_2 levels, etc. (Bale et al., 2002; Kiritani, 2006, 2007). Effects of climate change on insect herbivores can be direct, through impacts on their life history traits and number of generations per year (Gomi et al., 2007), phenology (Dingemanse and Kalkman, 2008), winter mortality (Ayres and Lombardero, 2000) and distribution range (Karban and Strauss, 2004), or indirect, e.g., when host-parasitoid interactions are affected (Menéndez et al., 2008) or when insects respond to climate-induced changes on the host plant (Forkner et al., 2008).

Knowledge on thermal tolerance is essential to predict the effects of climate change in an organism (Deutsch et al., 2008). Such information is not available for the coffee berry borer (CBB) *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae: Scolytinae) the most important pest of coffee throughout the world (Damon, 2000; Jaramillo et al., 2006). In this paper, we determine the thermal range for *H. hampei* and make some inferences on the effects of climate change on the pest and on coffee production in the tropics, using climatic data from four coffee producing area in Africa and South America. In addition, the original host plant and the possible area of origin of CBB as well as the reasons for the absence of the CBB in the presumed area of origin of *C. arabica* are discussed.

Material and Methods

Insects.

Females of the CBB were obtained from an *H. hampei* stock culture established in July 2005 with beetle-infested coffee berries collected from an organic coffee plantation located in South Kisii (Gucha), Western Kenya (0° 45' 49.85" S, 34° 43' 1.76" E). The colony is maintained at the International Center of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya, where the insects are reared on ca. 150 days old coffee berries (*C. arabica* var. Ruiru 11) kept at room temperature (25± 1°C), 70% ± 5% relative humidity [RH], and a 12:12 h (L: D) photoperiod. Infested berries were kept inside square plastic containers (40 x 40 x 20 cm) with perforated lids (55 mm diameter) covered with insect gauze. The bottom of each container was layered with a 1.5 cm mixture of plaster of Paris and activated charcoal to maintain

humidity and prevent the desiccation of the berries and the insects (Jaramillo et al., 2008).

Experimental setup and data assessment

The study was conducted at *icipe* laboratories. Organically produced coffee berries (*C. arabica* var. Ruiru 11) ca. 150 days old were collected from the Kiambu district of Central Kenya (1° 10' S, 36° 49' 60 E, 1,723 m.a.s.l.). Once in the laboratory, berries were surface sterilized using the protocol developed by Pérez et al. (2005): the berries were washed with detergent for 15 min, rinsed with tap water, then dipped in a 2% sodium hypochlorite solution for 10 min, rinsed again with sterile distilled water, t soaked in a 2% potassium sorbate solution and finally rinsed with sterile distilled water. Subsequently the excess of water was removed with a paper towel and the coffee berries were allowed to dry at room temperature. Afterwards, the berries were placed in round plastic containers (23 cm dia × 6.8 cm depth) and exposed to large numbers of *H. hampei* females from the stock culture. After 2h of exposure, berries that had been bored by one female were selected and transferred individually into each well of a 12-well microtiter plates (Costar® 3526, Corning Inc., Corning, NY, USA). Each well (23 mm dia; 20 mm deep) was filled with a 0.5 cm layer of a mixture of plaster of Paris and charcoal (Jaramillo et al., 2008). Twelve holes (15 mm dia), coinciding with the wells, were perforated in the lid of every multiwell plate and covered with mesh to allow aeration of the experimental units and to prevent escape of the beetles. The multiwell plates were then transferred to temperature controlled climate chambers (SANYO® MIR-553, Sanyo Electrical Ltd., Japan) set at eight different constant temperatures (15, 20, 23, 25, 27, 30, 33 and

35°C), 80 ± 5% RH, and a 12:12 h (L: D) photoperiod. To keep up the humidity inside the experimental units, distilled sterile water was added to each well every two, three days or daily for multiwell plates kept at 20-30°C, 15°C and 35-40°C, respectively.

Numbers of live and dead CBB colonizing females as well as their position inside the berries (see below), number of CBB life stages (i.e., eggs, larvae, prepupae, pupae and adults) were assessed daily for periods between 30 and 60 days depending on the temperature.

Four different positions based on the insect location within the berry have been identified by Bustillo et al. (1998) as follows: (A), when the female is starting to colonize a new berry but the penetration in the exocarp has not taken place; (B), when the female has penetrated the berry but has not yet reached the endosperm; (C), when the female has started to bore into the endosperm but not to oviposit; and (D), when the female has produced a gallery in the endosperm, and one or more of its immature stages are found inside the gallery.

The evaluations concluded when egg laying by the F2 generation was observed i.e., between 30 and 60 days after the infestation of the berries depending on the temperature. The coffee berries were dissected under a 10X stereomicroscope and the position of the colonizing female inside the berry was recorded and the numbers of CBB immature stages were counted. On a daily basis, five berries per temperature and per replicate were destructively sampled and dissected under the stereomicroscope. The experiment was repeated four times over time for insects kept at 15 and 25°C, and three times for insects kept at 20, 23, 27, 30 and 35°C.

Climatic data and estimated number of generations of Hypothenemus hampei *at four locations in Africa and South America*

Daily climatic data was obtained for four coffee growing areas, with three locations in East Africa (Jimma, Ethiopia; Kisii, Kenya; and Kilimanjaro, Tanzania) and one location in South America (Chinchiná - Colombia) (Fig. 5). Precipitation data was used to estimate the yearly blossoming period of the main coffee harvest in the different locations. A single heavy rain (>10 mm rain), followed by a prolonged dry period usually triggers the blossoming of a coffee tree (Trojer, 1986). *H. hampei* females start to search for suitable coffee berries around 100 days after flowering and oviposit inside the berries usually 20 days later (Ruiz, 1996). In the absence of CBB population dynamics data in all four locations, the findings of Trojer (1986) and Ruiz (1996) were used to estimate the probable time of CBB oviposition in the different locations in East Africa and Colombia. Therefore, long-term daily data on temperature (Fig. 5) and precipitation (data not shown) in the different locations, together with our laboratory derived data on degree-days for CBB, was used to estimate the number of potential CBB generations per year and location.

Warming Tolerance and Thermal Safety Margins of Hypothenemus hampei

Warming tolerance (average amount of environmental warming an ectotherm can tolerate before performance drops to fatal levels) and thermal safety margins (temperature at which the performance of the organism will start to decrease) were calculated according to Deutsch et al. (2008) as follows:

$$\text{Warming Tolerance (WT)} = CT_{max} - T_{hab}$$

and,

$$\text{Thermal Safety Margin (TSM)} = T_{opt} - T_{hab}$$

Where CT_{max} is the critical thermal maximum of CBB, T_{opt} is CBB's thermal optimum and T_{hab} is the current climatological temperature of the organism's habitat.

Statistical analysis

The mortality/survival and the positions of the colonizing CBB female inside the coffee berry (A, B, C and D) at each temperature were analyzed using logistic regression (PROC LOGISTIC; SAS, 1999).

Differences in developmental times, survivorship and life history parameters between temperatures were analyzed by analysis of variance (ANOVA), using the general linear model (PROC GLM; SAS, 1999). Percentages were transformed to arcsine values before analysis. The significance level was set at $P = 0.05$. For estimation of the lower developmental threshold (T_0) which is the intercept over the slope of the regression i.e., the numbers of day-degrees to complete the pre-reproductive phase and the thermal constant (Kc) which defined as one over the slope, a regression over the linear range of the relationship between temperature (T) and developmental rates [R (T)] of the insect was used (Campbell et al., 1974).

$$R(T) = a + b * T \qquad [1]$$

A modified Logan model (Logan et al., 1976) by Lactin et al. (1995) was used to describe the relationship between temperature and development rate,

$$R(T) = e^{\rho T} - e^{[\rho \tau max - (\tau max - T)/\Delta]} + \lambda \qquad [2]$$

Where e is the exponential function, T is the temperature in degrees Celsius (°C), ρ, Tmax, Δ and λ are fitted coefficients.

All parameters in nonlinear models were estimated by minimization of the sum of squared residuals. Parameters were tested against 0, based on non-overlap of 95% confidence intervals.

For Kilimanjaro (Tanzania), Kisii (Kenya), and Chinchiná (Colombia), the historical degree-days were calculated between 120 and 240 days after flowering. The number of degrees above the threshold degree-days, for a single day are calculated as follows:

$$\text{Degree-days (DD)} = 1/2*(\text{Max.} + \text{Min. temperature}) - T_0$$

Where Max. and Min. are daily maximum and minimum temperature (ºC). If Min. temperature was lower than the minimum threshold T_0, then Min. temperature was set to minimum threshold. If Max. temperature was higher than the maximum threshold 33ºC, then Max. temperature was set to 33ºC. The estimated number of CBB generations per year was calculated by dividing historical cumulative degree-days per year and location by the experimental estimation of *Kc*.

Life table statistics were calculated according to Hulting et al. (1990) using SAS, with calculation of confidence intervals for all estimated parameters (Maia et al., 2000). The two-sided *t*-tests values, as well as their respective *P* values were computed, and mean values were separated using a pairwise comparison between populations.

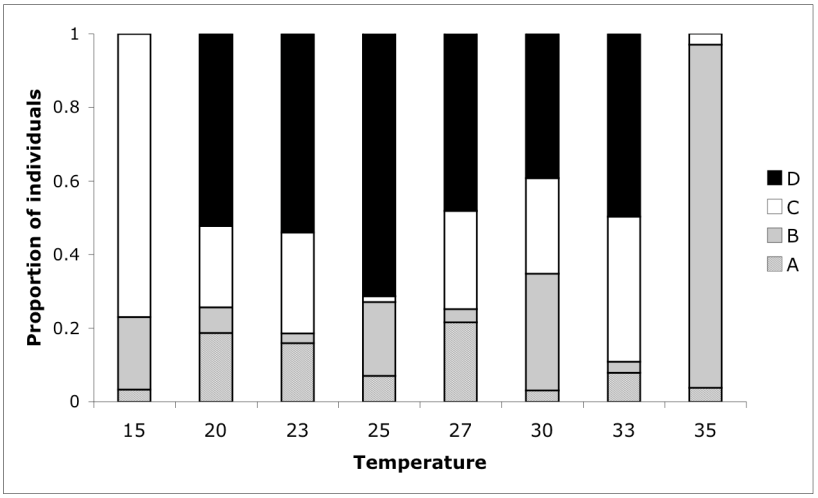
Results

Effect of temperature on the colonization of coffee berries and mortality by/of CBB colonizing females

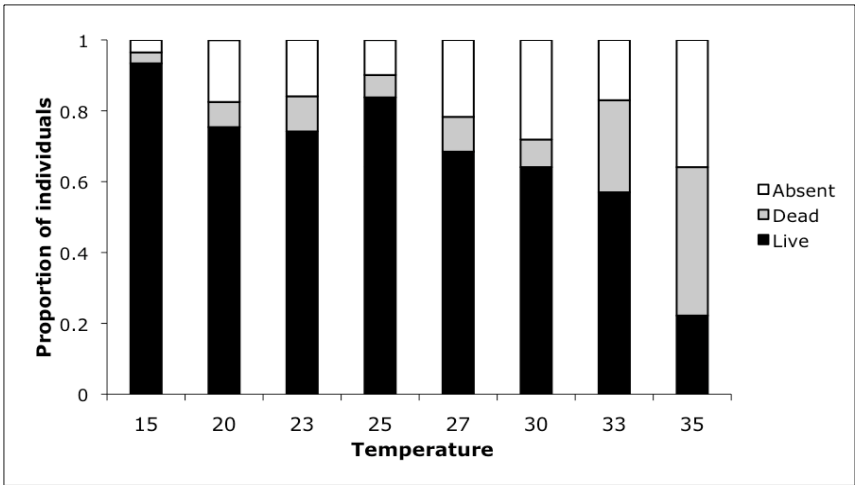
The proportion of colonizing CBB females in the different positions inside the berries and their mortality/survival as a function of temperature are presented in Fig. 1, respectively. Across temperatures, < 25 % of colonizing females failed to penetrate the berries (position A) (Fig. 1). The temperature significantly affected the mortality/survival and the position of the CBB females in the berries (χ^2 = 546.15, (7, N = 5099) $P < 0.0001$) and (χ^2 = 953.92, (7, N = 5099) $P < 0.0001$), respectively.

The highest proportion of CBB females found in position D was recorded at 25ºC (74.0 %), followed by 23ºC (54.2%), 20ºC (52.3 %) and 33ºC (49.7 %). At 15ºC and 35ºC there was no oviposition and the proportion of females found in the position B and C were 19.8%, 76.9% and 93.3%, 3.0%, respectively, indicating that at 15ºC the colonizing females reached the endosperm but did not oviposit. On the other hand at 35ºC the colonizing females did not reach the endosperm and remained in position B (Figure 1a).

The highest numbers of live females were found at 15ºC (93.4%), followed by 25ºC (83.8%) and 20ºC (75.4%) (Figure 1b). The highest numbers of dead CBB females were recorded at 35ºC followed by 33ºC (41.9% and 26.0%, respectively). In general, the proportion of surviving CBB females was high from 15 to 25ºC; at higher temperatures, survival started to decrease considerably (Figure 1b).



a



b

Figure 1. Proportion of coffee berry borer colonizing females (a) in the different positions in the berry; (b) live, dead or females that failed to penetrate the coffee berry.

Effect of temperature on the developmental rate of H. hampei

None of the CBB life stages developed successfully at 15 and 35ºC. The youngest life stages (egg and first instar larvae) developed between 20-33ºC, whereas second instar larvae, prepupa, pupa and adult developed only between 20-30ºC (Table 1). For all CBB life stages, the development time decreased significantly with temperature (between 20-30ºC for egg, larvae 1 and 2; and 20-27ºC for later life stages). At 33ºC females oviposited but subsequent dissections revealed that 95% of the L1 died after eclosion – mention here that there was no development for larva II onwards. The developmental time of CBB immature stages was significantly influenced by temperature, in particular the pre-pupal and pupal stages (Table 1). The duration of all immature stages except L1, was significantly longer at 20°C than at 23, 25, 27 and 30°C, i.e. for egg ($F = 29.51$, $P < 0.0001$), L2 ($F = 39.0$, $P < 0.0001$), pre-pupa ($F = 8.65$, $P = 0.0021$), pupa ($F = 22.40$, $P < 0.0001$). Egg to adult developmental time differed significantly at all temperatures tested ($F = 305.88$, $P < 0.0001$) (Table 1).

Table 1. Mean (±SE) developmental time (in days) of different life stages of *Hypothenemus hampei* at different constant temperatures.

Life stages	Temperature (ºC)							
	15*	**20**	**23**	**25**	**27**	**30**	**33****	**35***
Eggs	-	12.0 ± 0.6a	7.7 ± 0.3b	5.3 ± 0.3c	4.3 ± 0.3dc	3.3 ± 0.3d	4.7 ± 0.3dc	-
Larva I	-	6.3 ± 1.3b	3.3 ± 0.9c	2.8 ± 0.5c	2.0 ± 0.1c	1.7 ± 0.3c	9.0 ± 0.6a	-
Larva II	-	9.0 ± 0.6a	6.0 ± 0.6b	5.8 ± 1.1b	5.0 ± 0.6b	4.0 ± 0.6b	-	-
Pre-pupa	-	12.7 ± 0.7a	7.7 ± 1.2b	6.0 ± 0.4b	5.0 ± 0.6b	5.3 ± 1.2b	-	-
Pupa	-	16.3 ± 1.4a	6.5 ± 0.3b	6.3 ± 0.5b	5.2 ± 0.3b	6.0 ± 0.7b	-	-
Egg to adult	-	53.7 ± 0.7a	31.2 ± 0.4b	26.6 ± 0.5c	21.8 ± 0.3d	23.3 ± 0.3e	-	-

Within a row, means followed by the same letter are not significantly different (P= 0.05), SNK test. * CBB oviposition was not recorded at these temperatures. ** CBB oviposition took place at this temperature but the first instar larvae died after eclosion.

For all CBB life stages, significant relationships between the developmental rate and temperatures were recorded (Table 2). In egg, pre-pupa, pupa and egg to adult time the relationships were strongly linear (r^2= 0.91 P < 0.0001; 20-30ºC), (r^2= 0.75 P < 0.0001; 20-27ºC), (r^2= 0.66 P < 0.0001; 20-27ºC) and (r^2= 0.97 P < 0.0001; 20-27ºC), respectively, whereas a weaker relationship was recorded for L1 (r^2= 0.57 P = 0.0004; 20-30ºC). Linear regressions did not yield a good fit for development of the L2, therefore data are not presented in Table 2. For egg to adult, the lower developmental threshold was 14.9ºC and the thermal requirement for completion of the pre-reproductive phase was calculated as 262.47 degree-days above the lower developmental threshold (Table 2).

Table 2. Estimates of the linear regression analyses, lower thermal thresholds and the thermal constants for eggs, L1, pre-pupa, pupa and egg-adult of *Hypothenemus hampei*

Life stages	Linear range (ºC)	Regression Equations [a]	r^2	*F*	P>F	To[b]	Kc[c]
Eggs	20-30	Y = -0.37713+0.02265*T	0.91	152.91	< 0.0001	16.7	44.15
Larva I	20-30	Y = -0.78949+0.04815*T	0.57	21.05	0.0004	16.4	20.77
Pre pupae	20-27	Y = -0.27788+0.01791*T	0.75	38.08	< 0.0001	15.5	55.83
Pupa	20-27	Y = -0.29549+0.01861*T	0.66	50.46	< 0.0001	15.9	53.73
Egg-adult	20-27	Y = -0.05689+0.00381*T	0.97	861.15	< 0.0001	14.9	262.47

[a] Calculated after Campbell et al. (1974), where X is the temperature (°C) and Yis the developmental rate (1/developmental time). [b] Lower development threshold (°C). [c] Thermal constant (in day degrees).

Developmental rates increased linearly between 15 and 27°C for prepupa, pupa and adult and between 15 and 30°C for eggs and L1 (Fig. 2). In general, the non-linear model gave a good fit to the data sets within a range of 20-27°C for, and between 20-30°C for eggs and L1 (Figure 2).

The modified Logan model provided a good fit for the developmental rate data for all life stages (Fig. 2). The fitted parameters of the model are presented in table 3. Based on the non-linear models, the optimum temperature for the development of CBB egg and L1 was estimated as 30–32ºC and for L2, pre-pupa, pupa and adult between 27-30ºC. The lower and upper developmental threshold for all life stages was estimated as 14.9 and 30ºC, respectively (Figure 2).

Table 3. Fitted parameters of the non-linear modified Logan model (Lactin et al., 1995) for *Hypothenemus hampei* life stages

Life stages	Parameters				r^2	*F*	*P>F*
	ρ	T_{max}	Δ	λ			
Eggs	0.0153	35.5	1.198	-1.2806	0.90	75.51	<0.0001
Larva I	0.0254	34.9	0.1537	-1.4805	0.70	18.92	<0.0001
Pre pupae	0.0135	34.133	1.23	-1.2296	0.56	5.02	0.0176
Pupa	0.0374	43.7705	10.2205	-1.5557	0.63	15.76	0.0001
Egg-adult	0.00358	34.2548	0.1537	-1.0551	0.97	330.31	<0.0001

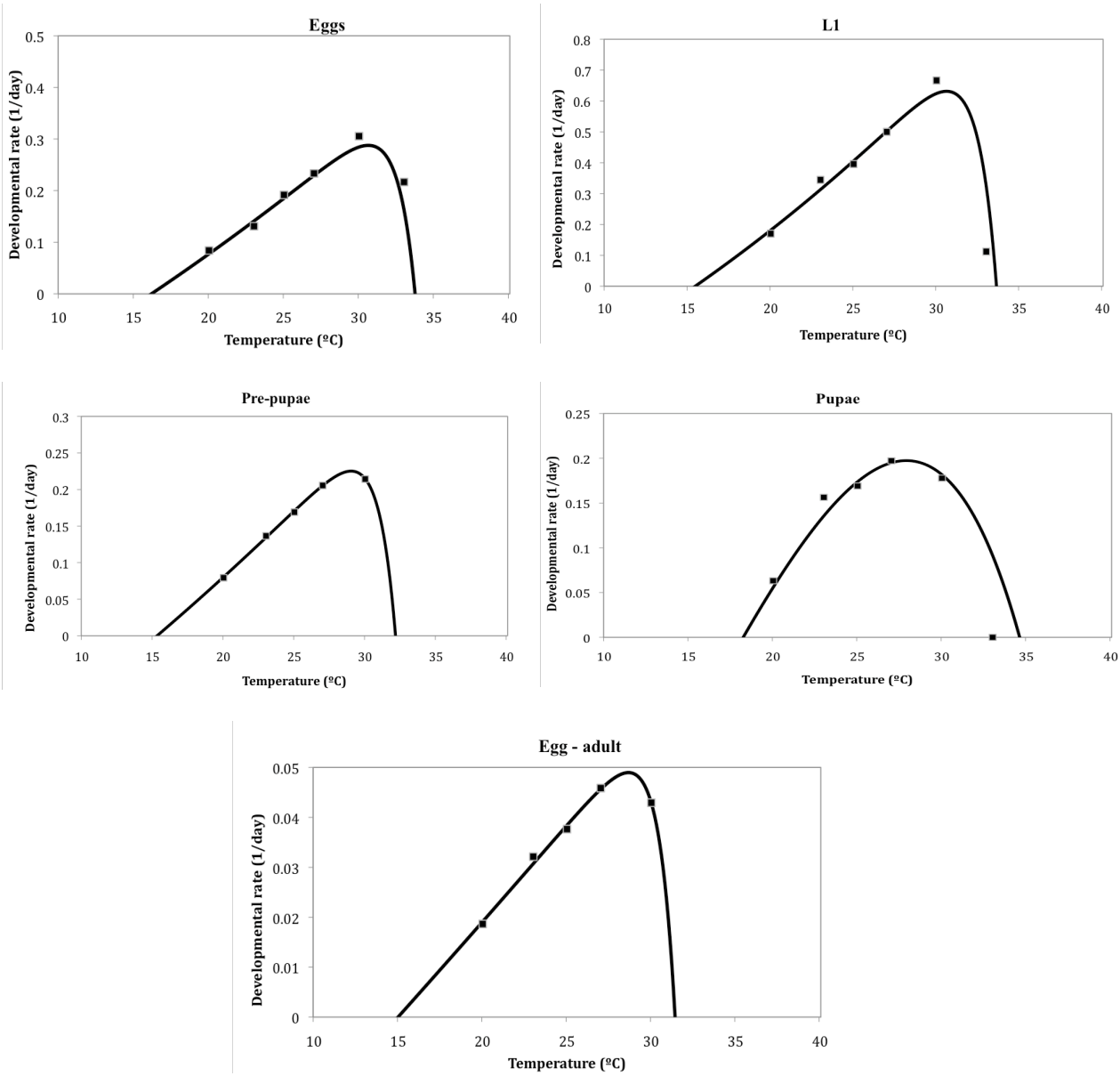


Figure 2. Effect of temperature on the developmental rates of *Hypothenemus hampei*

Life table parameters of H. hampei

Life table parameters are presented in Table 4. The intrinsic rate of increase (r_m) was significantly higher at 25 and 27°C. Similarly, the reproductive rate (R_0)

significantly differed among temperatures tested, and was highest at 25°C followed by 27°C. The lowest reproductive rate was recorded at 30°C. With 68.0 and 11.8 days, the maximum generation time (*G*) and the doubling time (*t*), respectively, were obtained at 20°C. The finite rate of increase (λ) remained almost constant at all temperatures tested (Table 4).

Table 4. Average (+SE) population growth parameters of CBB at five Constant temperatures

Parameter	Temperature (ºC)				
	20	**23**	**25**	**27**	**30**
r_m	0.06 ± 0.002a	0.10 ± 0.007a	0.14 ± 0.008ac	0.14 ± 0.0053ab	0.10 ± 0.028ad
R_0	54.0 ± 7.4a	67.9 ± 20.2a	146.6 ± 31.8a	84.5 ± 26.38ab	23.1 ± 12.9b
G	68.0 ± 1.2	40.9 ± 0.24	35.5 ± 1.0	32.76 ± 2.82	30.6 ± 0.8
λ	1.06 ± 0.002	1.10 ± 0.01	1.15 ± 0.009	1.14 ± 0.01	1.10 ± 0.031
t	11.8 ± 0.24	6.8 ± 0.45	4.9 ± 0.3	5.1 ± 0.2	6.8 ± 2.0

Means followed by the same letter within rows are not significantly different (P = 0.05, Student-Newman-Keuls sequential test). r_m,intrinsic rate of natural increase; R_0, net reproductive rate; G, mean generation time (days); λ, finite rate of increase; Dt, doubling time (days).

Effect of temperature on fecundity of H. hampei

Pre-oviposition and total CBB fecundity were significantly affected by temperature ($F = 8.08$, $P = 0.0035$) and ($F = 40.97$, $P < 0.0001$), respectively. The longest pre-oviposition periods were recorded at 20°C and 23°C (table 6). Total fecundity was significantly higher at 20°C (296.9 eggs) and lowest at 30°C (64.3

eggs) (table 5). No differences were recorded in sex ratio, which ranged from 0.84 to 0.9 for all temperatures tested.

Table 5. Mean (± SE) of pre-oviposition period, total fecundity, daily fecundity and sex ratio of *Hypothenemus hampei* at constant temperatures.

Parameters	Temperature (ºC)				
	20	**23**	**25**	**27**	**30**
Pre-oviposition period (days)	5.7 ± 0.3a	4.0 ± 0.0ab	3.3 ± 0.3b	3.7 ± 0.3b	3.0 ± 0.6b
Total fecundity*	296.94 ± 9.4a	199.6 ± 13.8b	201.5 ± 19.4b	160.0 ± 11.6b	64.3 ±8.4c
Sex ratio**	0.9 ± 0.07a	0.85 ± 0.03a	0.9 ± 0.004a	0.84 ± 0.2a	0.9 ± 0.1a

Means followed by the same letter within rows are not significantly different (P = 0.05, Student-Newman-Keuls sequential test). * Total number of eggs laid per female at a given temperature. ** Proportion of CBB females.

Mean daily fecundity of *H. hampei* females varied with temperature (Fig. 3). At 20°C the oviposition pattern of *H. hampei* was similar to 25°C and did not follow a distinct pattern. An uninterrupted oviposition period was recorded at 20°C from day 2 to 43 after infestation of the berries. After 43 days the oviposition fell to near zero, and from then on it started to peak again. This second peak of oviposition corresponded to eggs laid by the F1 females.

CBB females exposed to 23°C had two peaks of oviposition at around 18 and 22 days after infestation of the berry. The total oviposition period of the colonizing female at this temperature was around 28 days (Fig. 3b). Subsequently a smaller peak of oviposition recorded during days 34-40 may indicate the oviposition of F1 females. Figure 3c presents data for two generations (two peaks of oviposition) at 25°C. For the first generation, egg production was recorded from day 4 to 34 after infestation of

the berries. At this point the oviposition dropped basically to zero and the egg production of the F1 took place from days 35 to 61. Overall, egg production at 27°C was low, and occurred over a period of around 30 days (Fig. 3d). Lowest egg production and over the shortest period of time was recorded at 30°C (Fig. 3e).

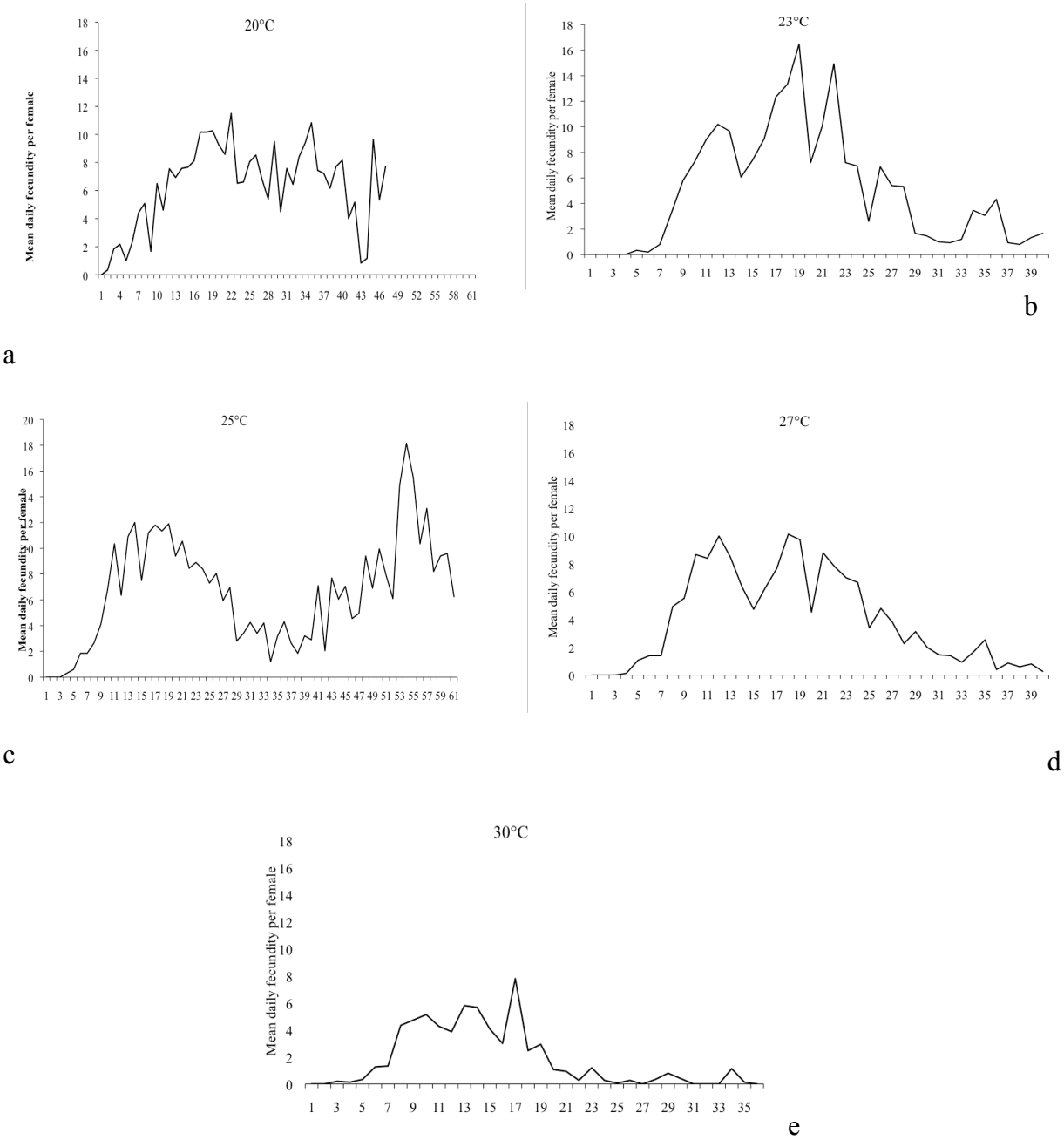
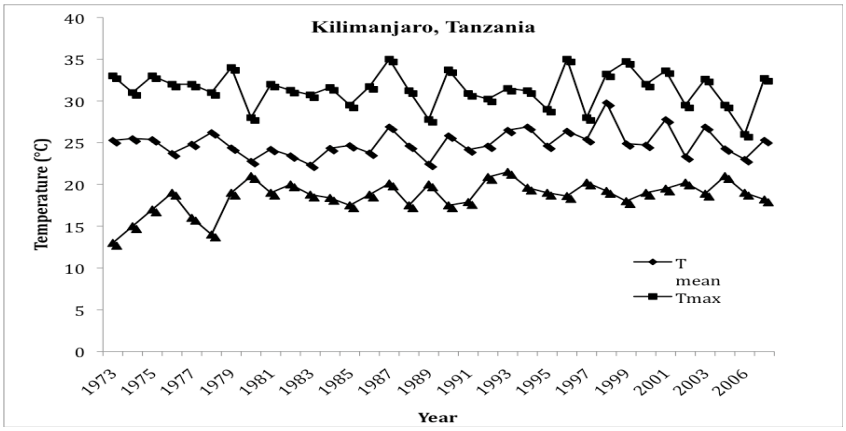
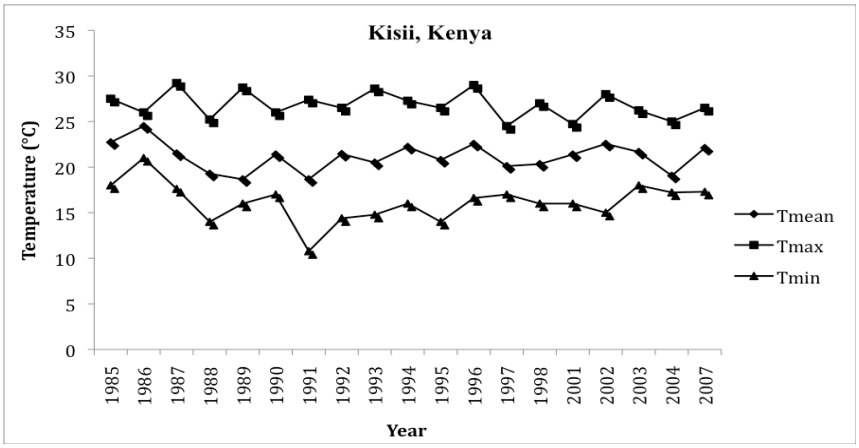


Figure 3. Egg laying pattern of *Hypothenemus hampei* females in berries kept at 20°C, 23°C, 25°C, 27°C, 30°C.

Estimated number of Hypothenemus hampei *generations in four coffee growing locations in East Africa and South America*

Temperature data for the four locations are presented in Figs. 4a-d. The number of CBB generations per year could not be estimated for Jimma (Ethiopia), due to a mean minimum temperature below < 15°C throughout the entire year (Fig. 4c); the lowest thermal threshold of CBB under laboratory conditions calculated in this study was 14.9°C.





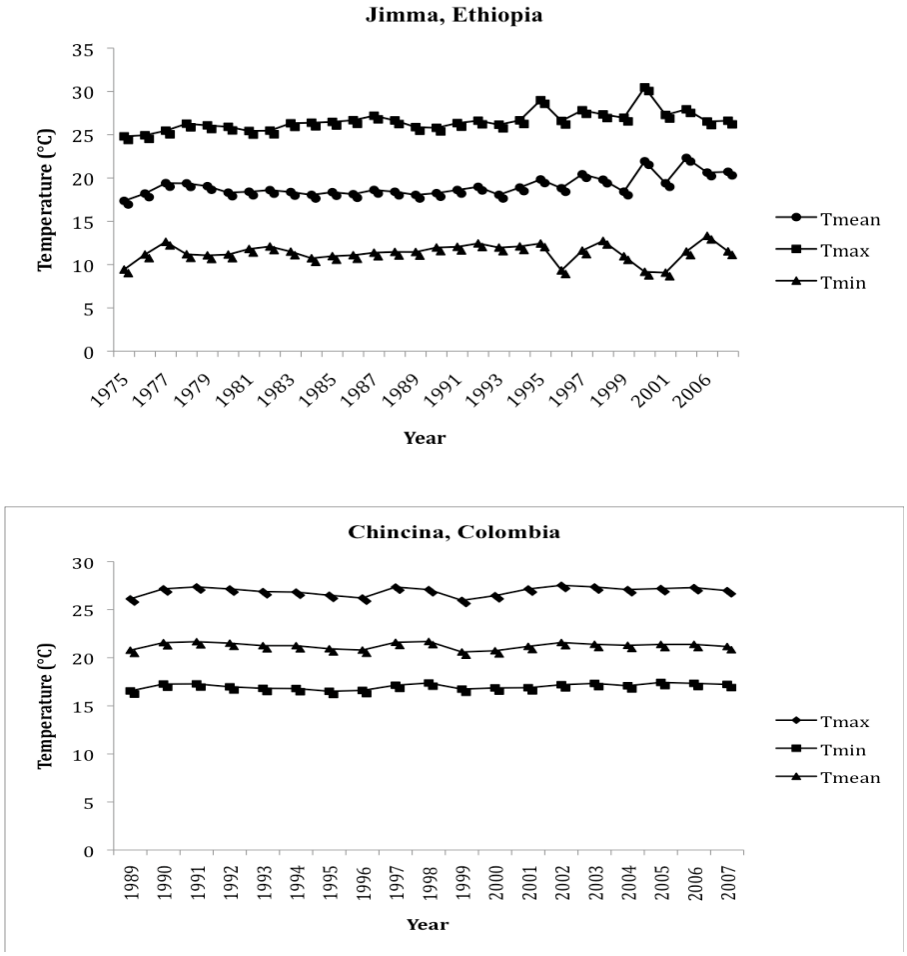


Figure 4. Mean yearly temperature for Kilimanjaro, Tanzania; Kisii, Kenya; Jimma, Ethiopia, and Chinchiná, Colombia.

Based on the number of degree-days in the 3 sites in Colombia, Kenya and Tanzania (Table 6), the estimated number of generations of *H. hampei* per year ranged from 2.03 to 4.71. The calculated number of CBB generations for Kilimanjaro and Chinchiná were very similar, ranging from 2.39 to 4.71, and 2.95 and 4.30, respectively. With 2.03-3.13 the lowest number of beetle generations per year was estimated for t Kisii, Kenya (Figure 5).

Table 6. Number of degree days available to *Hypothenemus hampei* (above 14.9°C) in two locations in East Africa and one in South America.

Year	Location		
	Chinchiná, Colombia	**Kilimanjaro, Tanzania**	**Kisii, Kenya**
1974	-*	688.42	-**
1975	-*	838.51	-**
1976	-*	875.73	-**
1977	-*	927.20	-**
1978	-*	821.45	-**
1979	-*	667.73	-**
1980	-*	-**	-**
1981	-*	851.40	-**
1982	-*	688.10	-**
1983	-*	824.64	-**
1984	-*	832.60	-**
1985	-*	773.71	587.44
1986	-*	709.32	582.50
1987	-*	995.70	633.68
1988	-*	823.71	637.97
1989	775.01	846.90	673.85
1990	906.35	676.61	533.85
1991	1128.68	746.59	554.74
1992	888.85	712.28	593.23
1993	841.40	-**	726.17
1994	848.81	-**	772.98
1995	949.74	-**	823.15
1996	1002.68	-**	774.59

1997	946.24	628.37	828.34
1998	896.65	706.69	647.23
1999	594.88	-**	-**
2000	833.90	799.04	-**
2001	877.90	825.56	-**
2002	921.54	1082.46	636.19
2003	885.65	799.80	769.26
2004	861.50	862.86	672.84
2005	886.80	-**	-**
2006	941.64	1237.57	-**
2007	876.20	982.02	639.25

* CBB not yet present in this coffee growing area of Colombia. ** Climatic data not available. Either the blossoming period of the coffee plants or the number of degrees days could not be estimated for these years.

Data on warming tolerance and thermal safety margins of CBB for the two East African locations showed considerably high variability compared to the Colombian site (Table 7). Moreover, both indices were substantially lower in the African locations, indicating higher vulnerability of the pest to climatic changes in this region compared to Chinchiná Colombia.

Table 7. Warming tolerance (WT) and thermal safety margin (TSM) (calculated after Deutsch et al., 2008) for *Hypothenemus hampei* in two locations in East Africa and one in South America.

Year	Location					
	Chinchiná, Colombia		Kilimanjaro, Tanzania		Kisii, Kenya	
	WT	TSM	WT	TSM	WT	TSM
1974	-*	-*	4.50	1.50	-**	-**
1975	-*	-*	4.60	1.60	-**	-**
1976	-*	-*	6.27	3.27	-**	-**
1977	-*	-*	5.16	2.16	-**	-**
1978	-*	-*	3.77	0.77	-**	-**
1979	-*	-*	5.61	2.61	-**	-**
1980	-*	-*	7.22	4.22	-**	-**
1981	-*	-*	5.77	2.77	-**	-**
1982	-*	-*	6.55	3.55	-**	-**
1983	-*	-*	7.66	4.66	-**	-**
1984	-*	-*	5.66	2.66	-**	-**
1985	-*	-*	5.33	2.33	7.27	4.27
1986	-*	-*	6.22	3.22	5.49	2.49
1987	-*	-*	3.11	0.11	8.49	5.49
1988	-*	-*	5.38	2.38	10.72	7.72
1989	9.21	6.21	7.55	4.55	11.33	8.33
1990	8.43	5.43	4.16	1.16	8.60	5.60
1991	8.33	5.33	5.83	2.83	11.33	8.33
1992	8.48	5.48	5.38	2.38	8.55	5.55
1993	8.74	5.74	3.50	0.50	9.49	6.49
1994	8.74	5.74	3.10	0.10	7.72	4.72

1995	9.06	6.06	5.38	2.38	9.22	6.22
1996	9.20	6.20	3.60	0.60	7.44	4.44
1997	8.39	5.39	4.61	1.61	9.88	6.88
1998	8.30	5.30	0.22	-2.78	9.66	6.66
1999	9.40	6.40	5.11	2.11	-**	-**
2000	9.27	6.27	5.27	2.27	-**	-**
2001	8.81	5.81	2.22	-0.78	8.60	5.60
2002	8.41	5.41	6.66	3.66	7.44	4.44
2003	8.60	5.60	3.11	0.11	8.33	5.33
2004	8.70	5.70	5.72	2.72	10.94	7.94
2005	8.61	5.61	-**	-**	-**	-**
2006	8.61	5.61	6.98	3.98	-**	-**
2007	8.83	5.83	4.7	1.7	7.9	4.39

* CBB not yet present in this coffee growing area of Colombia. ** Climatic data not available. Either the blossoming period of the coffee plants or the number of degrees days could not be estimated for this years.

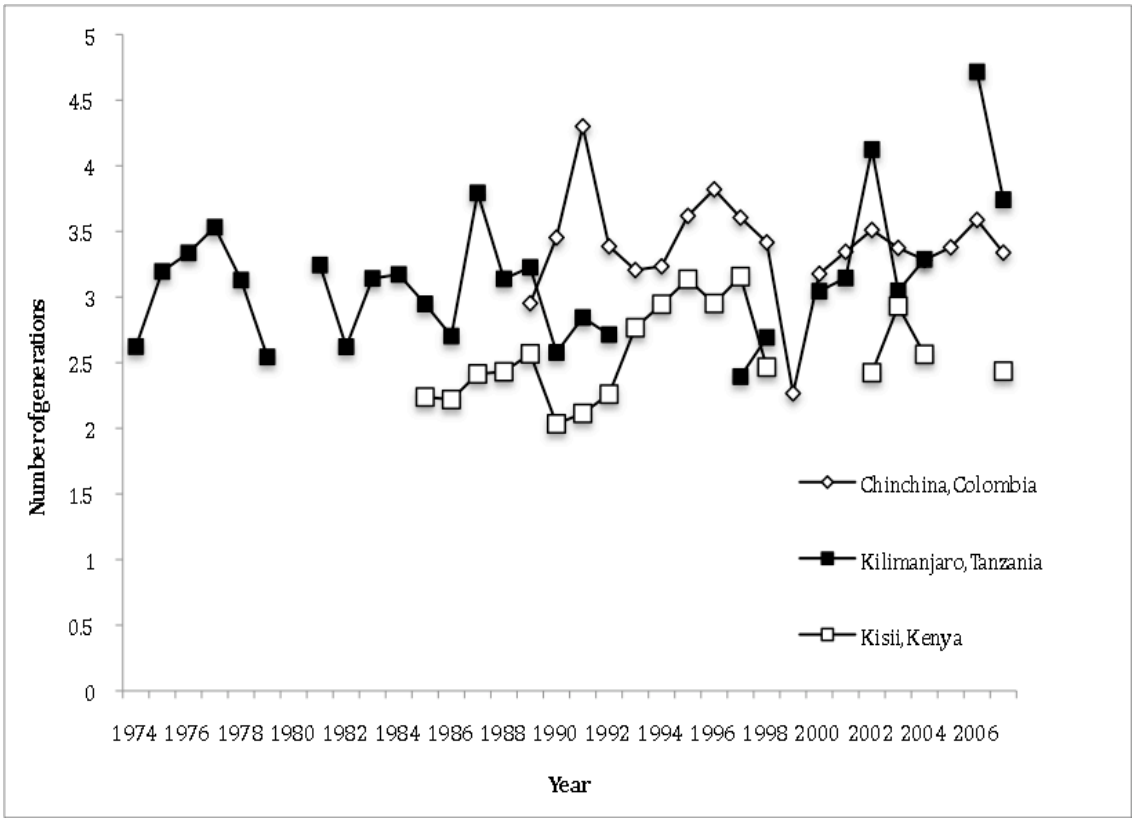


Figure 5. Number of generations of *Hypothenemus hampei* in study sites in Colombia, Tanzania, and Kenya.

Discussion

Global warming is already affecting the bionomics of arthropods. Detailed studies from the temperate zones on several species report mainly positive effects on insect fitness and distribution range (Parmesan et al., 1999; Bale et al., 2002; Gomi et al., 2007; Musolin, 2007; Dingemanse and Kalkman, 2008). However, in a recent paper Deutsch et al. (2008) predict negative effects of global warming on tropical arthropods. Based on IPCC (2007) predictions, tropical arthropods are at risk of extinction because of their narrower thermal tolerance. Surprisingly, the

potential impact of global warming in tropical insects has only been studied in hematophagous insects such as malaria-transmitting *Anopheles* spp. (Diptera: Culicidae) (Patz and Olson, 2006) and the tsetse fly *Glossina pallidipes* (Diptera: Glossinidae) (Terblanche et al., 2008). In this paper, we report the first detailed analysis on the potential effects of climate change on the bionomics of the CBB, the most devastating pest of coffee throughout the world.

Because of the absence of a standardized methodology to investigate CBBs in the laboratory using coffee berries as a substrate, previous studies on the basic biology of the insect were either carried out in the field (Borbón-Martinez, 1989; Baker et al., 1992; Ruiz, 1996), or in the laboratory with only one temperature regime (e.g., Romero and Cortina, 2004, 2007; Fernandez and Cordero, 2007). Using a recently developed experimental protocol (Jaramillo et al., 2008) we were able to study the effects of seven different temperature regimes on the bionomics of the CBB. According to Steven's (1989) climatic variability hypothesis the thermal tolerance of an insect is directly proportional to the climatic variability the organism is exposed to. The narrow range of temperatures within which *H. hampei* can develop confirms this hypothesis. The extremes for CBB survival are 15 and 30°C, but development takes place only between 20 and 30°C. Although our model predicts fastest development of CBB between 27-30°C, there is clear trade-off between development time and reproductive success as previously shown for other insects (Roff, 2000). Interestingly, the highest rate of survival in colonizing females was recorded at the lowest temperature tested (Table 1).

CBB attacks and successfully develops on both *C. arabica* and *C. canephora* (Le Pelley, 1968). Moreover, CBB is the only herbivore feeding on the

endosperm of coffee due to its ability to detoxify caffeine (Vega et al., 2003b, Vega 2004). *C. arabica* is believed to have originated in the south-western highlands of Ethiopia where it naturally grows as an understory tree in forests (Davis et al. 2006). In this area, mean air temperatures range between 18-22°C (Alegre, 1959). In contrast, *C. canephora* is native to the lowland forests of the Congo River Basin where mean annual air temperatures are 24-26°C, (Coste, 1993). For many years, there has been controversy in the literature about the geographic origin of the pest (Bergamín, 1943; Ticheler, 1961; Benavides et al., 2005) and its original host plant(s) (Baker, 1984; Davidson, 1967). Resolving this mystery might have important implications for future breeding for host plant resistance in coffee, as well as better targeted explorations for natural enemies of CBB. Based on our estimates on the thermal tolerance of CBB it is obvious that the insect cannot develop in the area around Jimma (Ethiopia) due to the low mean annual minimum temperatures prevalent there. During an extensive survey, Davidson (1967) did not find the CBB in Ethiopia and Damon (2000) later speculated that the absence of the pest is due to either specialized natural enemies, resistant varieties of *C. arabica* or exceptionally clean harvest practices in Ethiopian plantations. Yet, in a recent study Mendesil et al. (2004) reported widespread occurrence of CBB in southwestern Ethiopia. Since we were only able to obtain meteorological data from Jimma we speculate that climatic conditions in other parts of southwestern Ethiopia are more conducive for the development of the insect, explaining Mendesil's et al. (2004) field observations.

According to Hodkinson (1999), distribution and range limits of an insect can be fully explained by physiological data linked to microclimatological

measurements, and Campbell et al. (1974) emphasized the usefulness of the lower threshold of development and the thermal constant of an insect to elucidate its potential distribution. Similar to *C. arabica, C. canephora* is an understory tree of lowland forests. Climatological data from shaded coffee plantations in Central America (Barradas and Fanjul, 1986; Vaast et al., 2004) and East Africa (Kirkpatrick, 1935) indicate a reduction in temperature between 2-6°C depending on the region, when compared to coffee grown without shade. Considering these findings with our data on thermal tolerance of CBB and the annual mean temperatures of the Congo River Basin, the presumed area of origin of *C. canephora* (Davis et al., 2006), we hypothesize that the original host plant of *H. hampei* is likely to be robusta coffee. The geographic distribution of *C. arabica* and *C. canephora* in Central and Eastern Africa overlap to a certain extent (Davis et al. 2006). *C. canephora* has higher caffeine content than *C. arabica* (Spiller 1998) and CBB is more attracted to this species than to 6 other species (Guerreiro Filho and Mazzafera, 2003). Based on these findings the authors conclude that *H. hampei* has evolved an adaptation to handle the toxic effects of caffeine. Extending the host range to a related plant species that produces considerably less secondary plant metabolites increases the fitness of the herbivore (Rosenthal and Berenbaum, 1992). Thus, in the absence of data comparing the performance of CBB on *C. arabica* and *C. canephora* we additionally hypothesize that *H. hampei* extended its original host range to *C. arabica* because of the spatial proximity of the two *Coffea* spp. and possibly also because of increased fitness on a host plant where the beetle needs to detoxify considerably less caffeine.

How do the findings reported in this paper relate to potential climate change effects on the beetle? In a review Bale et al. (2002) suggest that direct effects of temperature are likely to be stronger and more important than other factors related to climate change such as CO_2 levels, rainfall pattern, etc. Moreover, data on thermal tolerance of an insect is crucial to predict the possible effects of climate change (e.g., Hodkinson et al., 1999; Kiritani, 2007; Gomi et al., 2007; Calosi et al., 2008). The climatic data from the four locations in Colombia, Tanzania, Kenya and Ethiopia used in this study do not show an increase in temperature during the observation period. This might be due to the length of the observation period in the case of Colombia, and the quality of the data, with major gaps in all three data sets from East Africa. According to our estimations for the Colombian, Tanzanian and Kenyan sites (as mentioned before, the climate data from Jimma, Ethiopia indicated no development of CBB in this area), the potential number of CBB generations per year fluctuates between 2 and 4.5. However, laboratory data indicates that the carrying capacity of an individual *C. arabica* berry does not exceed 3 generations (J. Jaramillo, unpubl. data). According to our data on thermal tolerance of CBB and the calculations of warming tolerance and thermal safety margins using the formulas proposed by Deutsch et al. (2008), a small increase in temperature will lead to a considerably faster development of the pest. Since the coffee berry is a finite resource, this will lead to an increased CBB dispersal, as more females will be competing for oviposition sites. Such a scenario will have devastating effects in coffee growing areas like Colombia where, because of well distributed precipitation leading to multiple flowering of the plants, there is yearlong supply of coffee berries (Arcila et al., 1993). The problem will be less severe in East Africa where there is a

marked and prolonged dry period, with consequent absence of berries in the field for extended periods.

Specialist herbivores do not have an adaptation capacity as great as generalist herbivores under climate change (Ward and Masters, 2007), and according to Hodkinson (1999) the eco-physiology of both insect and plants will predict the future distribution of insect pests when both host plant and herbivore are in close synchrony. Thus, in the case of a highly specialized herbivore like *H. hampei*, the effects of climate change on the insect and the plant cannot be separated. Assad et al. (2004) for Brazil, Gay et al. (2006) for Mexico and Grid (2002) for Uganda predict that even a small increase in temperature due to climate change, will have serious consequences for coffee production in these countries, in some cases rendering production very difficult. Under a climate change scenario, species like *H. hampei*, whose distribution appears to be restricted by their host plants, will follow them as they extend their range (Hodkinson, 1999; Ward and Masters, 2007). Coffee is mainly grown in the tropics from 20-25°N to 24°S (Wellman, 1961). Strategies that insects would use to cope with climate change include, among others, acclimation (Wilson and Franklin, 2002; Terblanche et al., 2005), and changes in latitudinal and altitudinal distribution (Neuvonen et al., 1999; Gaston and Chown, 1999; Menendez et al 2008). A latitudinal expansion in *C. arabica* and *C. canephora* is problematic because both species are highly susceptible to changes in photoperiod, with effects ranging from a marked reduction of their growth phase to an inhibition of flower development (Amaral et al., 2006). Yet to date coffee is grown in, among others, Nepal (http://www.plantecnepal.com/) and the Yunnan province of China (http://www.yunnancoffee.org/), both areas

outside the before mentioned tropical distribution range of *C. arabica* and *C. canephora*. Our data on thermal tolerance of *H. hampei* on the other hand would predict that the pest is well capable to thrive also under such conditions. An altitudinal expansion as a coping strategy in a climate change environment is potentially feasible, though there are few areas in the tropics where coffee production could expand in altitude, e.g. the Kilimanjaro area of Tanzania, Mount Kenya, and the mountain ranges of Colombia, considering that other requirements for successful coffee production like soil type and appropriate rainfall patterns have to be met (Damatta et al., 2008). For instance, Jaramillo (2005a) estimated that for the coffee growing area in Colombia an increase in temperature of 1°C would require to move the plantations by 167m in altitude to maintain the same productivity and quality in arabica coffee.

A proven strategy to alleviate the potentially negative effects of climate change on coffee production is the introduction of shade trees in coffee plantations (Lin, 2007; Damatta, 2008). Shade trees mitigate microclimatic extremes and can buffer coffee plants from microclimate variability (Beer et al., 1998), reduce high solar radiation and buffer detrimental diurnal changes in air temperature and humidity (Vaast et al., 2004; Vaast et al., 2006; Lin, 2007), leading to a decrease in the temperature around the coffee berries by up to 4°C (Jaramillo, 2005b) under low altitude conditions (i.e., < 700 m.a.s.l.), and by up to 2°C under mid to high altitude conditions (i.e., > 1,100 m.a.s.l.) (Vaast et al., 2004; Vaast et al., 2006). Moreover, Teodoro et al. (2008) recently demonstrated that densities of CBB were significantly lower in shaded versus un-shaded coffee plantations, possibly because shade coffee agro-ecosystems can serve as a refuge for beneficial arthropods (native and

introduced), leading to higher levels of biological control of *H. hampei* (Perfecto et al., 1996; Tylianakis et al., 2005). Finally despite lower yields of shaded compared to un-shaded coffee, the berry weight is higher and the quality of coffee produced under shade is better (Muschler et al., 2001), with overall favorable the economics for small-scale producers look (Gordon et al., 2007).

Our results indicate that the current prospects for climate change could severely threaten coffee production, not only due to the direct effects of temperature on the plant, but also to the possibility of increased yield losses due to the expanded geographical range of *H. hampei*, the most important pest of coffee. Thus we believe the most appropriate way for coffee production systems to cope with climate change is to come back to the origins of coffee as an understory tree in the forests of Africa.

General discussion

Around 70% of the world's coffee is produced by small-scale farmers, with over 20 million coffee-farming families – equivalent to more than 100 million people depending on its production for their subsistence (Vega et al., 2003a).

The main threat to coffee production and the livelihoods of these millions of families is the coffee berry borer (CBB) *H. hampei* (Damon, 2000; Jaramillo et al., 2006 [**Chapter 1**]). Infestation levels of the insect can be as high as 90% (Vega, 2004), and annual losses worldwide exceed US $500 million. The pest was first described in 1867 in France feeding on coffee beans of unknown origin (Waterhouse and Norris, 1989). Since then *H. hampei* has spread to all coffee producing countries worldwide except Hawaii (Vega, 2004). So far three parasitoids of African origin, the larval-pupal ectoparasitoids *P. nasuta* and *C. stephanoderis*, and the adult parasitoid *P. coffea*, have been introduced for biological control of CBB in several outbreak countries. They have been used both as classical biological control agents as well as in an augmentative manner.

Phymastichus coffea appeared to be a very promising candidate due to its ability to attack, paralyze and kill CBB females before they penetrate into the endosperm, thereby preventing economic losses (Gutierrez et al., 1998). In our studies with *P. coffea* under field conditions in Colombia (cf. Chapter 2) we found that levels of CBB parasitism approached 85% following augmentative releases of the parasitoids. This suggests that *P. coffea* had a strong impact on its host. 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. Four different positions based on the insect location within the berry have been identified by Bustillo et al. (1998): (A), when the female is starting to colonize a new berry but the penetration in the exocarp has not taken place; (B), when the female has penetrated the berry but has not yet reached the endosperm; (C), when the female has started to bore into the endosperm but not to oviposit; and (D), when the female has produced a gallery in the endosperm, and one or more of its immature stages are found inside the gallery. We recorded highest levels of parasitism in beetles found in position C of 150 days old berries independent of the time of the parasitoid releases. At this time most of the parasitized CBB had just begun damaging the endosperm without oviposition. After parasitization by *P. coffea* CBB females stop ovipositing and their mobility is impaired (Feldhege, 1992; Infante et al., 1994). In 90 days old berries when *P. coffea* was released one day after the artificial CBB infestation around 60% of the parasitized beetles were found in position B. Yet when the parasitoids were released five or nine days after the CBB infestation, highest levels of parasitism were recorded in CBB 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* L. (Coleoptera: Scolytidae) and *Tomicobia seitneri* (Ruschka) (Hymenoptera: Pteromalidae), where parasitized beetles continued to bore into the bark (Sachtleben, 1952). Likewise Feldhege (1992) observed that CBB 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 CBB females in positions C or D (Borbón, 1989; Infante et al., 1994; Lopez and Moore, 1998). Thus the high levels of parasitism recorded in 90 and 150 days old berries might be

due to the long time CBB 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* as illustrated by the low levels of parasitism in CBB in 210 days old berries recorded in our studies. At this stage 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, *H. hampei* females 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 CBB in position D in 210 days old coffee berries recorded in this study.

Our results show that *P. coffea* is a promising candidate for augmentative biological control of CBB although its control potential decreases with the age of the coffee berries.

In a parallel study we determined the extent of superparasitism in *P. coffea* under field conditions in Colombia (cf. **Chapter 3**). Considerable levels of superparasitism were recorded, depending on the age of the coffee berries, the positions of the beetles inside the berries and the time of parasitoid releases. In the field, various factors such as patch quality (van Alphen and Visser, 1990), food availability (Harvey et al., 2001), and the physiology of the female parasitoid, including its egg load (Babendreier and 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 CBB. We believe that physical effects of the berries, as a result of their dry matter content, are the main factors 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 CBB in the coffee berries, and thus the availability of hosts for *P. coffea* (Salazar et al., 1993; Ruiz, 1996; Jaramillo et al., 2005).

In this study, in the same way as parasitism, superparasitism, either self or conspecific, was recorded when *P. coffea* were released at a time when *H. hampei* had just commenced penetrating the coffee berries (positions A and B), and were 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 and Moore, 1998; **Chapter 2**). 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 and 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 in 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, 1996). 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 (**Chapter 2**). Effects of host plants on natural enemies have been extensively studied. Host plant traits such as morphology, plant nutrition, leaf mineral content (Jiang and 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. Theoretical models predict that when the patch is depleted, i.e., when unparasitized hosts become less frequent, superparasitism becomes an adaptive strategy (van Alphen and 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 meters' distance from the parasitoid release point in a commercial coffee plantation in Colombia. The results of **Chapter 3** 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 *H. hampei* by *P. coffea* under fields conditions.

In conclusion, Chapters 2 and 3 show that for future mass releases of the *P. coffea* in coffee plantations, in addition to an optimisation of the host-parasitoid release rates, the physiological state of the coffee berries at the time of releases, which can be determined by recording the major blossoming period of coffee plants (Bustillo et al., 1998), has to be considered to avoid superparasitism and to achieve the greatest possible CBB control in the field.

As mentioned before so far only four specialized natural enemies, all of them parasitoids, have been identified in the aboriginal home of *H. hampei*. Thus the potential discovery of additional natural enemies of CBB in Africa cannot be ruled out. Hence since October 2006 field explorations in the Kisii district or area ? of Western Kenya were carried out, exactly where the original collections of *P. coffea* that lead to the later introduction to Colombia were made (R. Mugo, Coffee Research Foundation, Ruiru, Kenya, Pers. Comm.).

Two years of rigorous sampling revealed a surprisingly low diversity of natural enemies of CBB. Apart from the bethylid parasitoid *P. nasuta*, the by far dominating natural enemy of CBB in our study, and its ceraphronid hyperparasitoid *Aphanogmus* sp. (Chapters 4 and 5), all other known parasitoids of *H. hampei* were either absent or recorded in very low numbers. Especially *P. coffea* was virtually nonexistent in our sampling area during the time of the study. This may be an indication that the parasitoid is not an important CBB mortality factor, at least in the Kisii area, as previously thought. Likewise the braconid *H. coffeicola* was never found during our two years of sampling, though in a similar study in neighbouring Uganda, Hargreaves (1926) reported *H. coffeicola* and *P. nasuta* as the two predominant natural enemies of CBB. Out of the 10,342 CBB parasitoids identified in

the present study only two specimens of *C. stephanoderis* were obtained. Vega et al. (1999) recorded *C. stephanoderis* as the most prevalent CBB parasitoid in Togo, and 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.

In agreement to observations by Hargreaves (1926) in Uganda, we recorded one major *P. nasuta* emergence peak (in February to May) in Kisii. *Aphanogmus* sp. started to appear approximately one month later. This is the first report of a hyperparasitoid of a primary parasitoid of *H. hampei* in Africa. It spends most of its time inside the coffee berries within the CBB galleries. Before parasitizing *P. nasuta*, *Aphanogmus* sp. probes with its antennae the older host larvae or pupae just before construction of the cocoons. Around 10% of the total number of *P. nasuta* cocoons were parasitized by its hyperparasitoid. *Aphanogmus* sp. usually oviposits on the abdomen of *P. nasuta*, and up to three larvae or pupae were found inside the *P. nasuta* cocoons.

A possible 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 plantations were not shaded and were 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? For instance 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 following 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 (Perez-Lachaud et al., 2002; Perez-Lachaud et al., 2004; Batchelor et al., 2005; Batchelor et al., 2006). However, Batchelor et al. (2006) recently suggested that *P. nasuta* should be the most effective biological control agent of CBB in the field due to its comparatively superior emergence rate and female offspring production.

In our study, out of the 8,893 *P. nasuta* individuals collected, 2.7% emerged from berries picked from the trees, whereas 97.3% originated from berries that were collected on the ground. Likewise, the parasitism rates by *P. nasuta* in berries that were collected on the ground ranged between 17.8 and 47.1%, whereas in the berries sampled in the trees parasitism rates were negligible to the extent that could not be calculated. A possible explanation for differences in parasitism rates in both strata e.g., ground and tree, may be an attack by *P. nasuta* in berries that are very close to the harvest point, which under natural conditions fall down to 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 *H. hampei* 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). Berries harbouring the pest 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). Our data from Western Kenya clearly shows that the coffee berries on the ground are not only the main reservoir of *H. hampei* 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* and consequently the biological control of CBB in the Americas, as the parasitoid spends most of its life cycle within the coffee berries (Hargreaves, 1935; Abraham et al., 1990).

The positive effects of cultural control on biological control agents have extensively been studies (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 in some arable crops. In the process of sugar beet cultivation during ploughing, the life stages of a pteromalid parasitoid of the Eastern sugar-beet weevil *Bothynoderes punctiventris* Germ (Coleoptera: Curculionidae) are shifted to the deeper soil layers and subsequently cannot reach the surface, rendering the parasitoid ineffective against the pest (van den Bosch and Telford, 1964). Furthermore, the biological control of the cereal leaf beetle *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae) is also affected by ploughing. The beetle usually overwinters as adult outside the field, however its larval and pupal parasitoids do so in the soil of the field, and as a result are destroyed during ploughing (van Emden and Service, 2004). In a similar way, parasitism rates of the pollen beetle *Meligthes aeneus* F. (Coleoptera: Nitidulidae) by two ichneumonid larval parasitoids, *Phradis interstitialis* Thomson and *Tersilochus heterocerus* Thomson are severely affected by ploughing (Williams, 2006). Like the parasitoids of the cereal leaf beetle, they also overwinter in the soil of the rape field

and emerge in the following spring. Therefore, ploughing substantially reduces their survival. For example, Ferguson et al., 2003 in their study could demonstrate that from 24% of *M. aeneus* larvae that were parasitized, only less than 2% of the parasitiods managed to survive the overwintering period.

Another example comes from a recent study showing that reduced tillage is associated with increased abundance of the carabid slug predator *Notonomus gravis* (Chaudoir) in arable crops (Nash et al., 2008). Finally pruning can also affect biological control as shown for the Antestia bug *Antestiopsis orbitalis* Westwood (Hemiptera: Pentatomidae), a serious pest of coffee in East Africa (Cilas et al., 1998). Pruning is used to diminish humidity in the coffee plantation; however, the parasitoids of antestia bugs also suffer when humidity is low (H.F. van Emden, pers. comm.).

Yet, two strikingly similar examples to CBB and *P. nasuta* have been reported for fruit flies in Hawaii and the horse chestnut leafminer, *Cameraria ohridella* Deschka and Dimic (Lepidoptera, Gracillariidae) in Europe. 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. Similarly, Kehrli et al (2005) found that the parasitoids of the horse chesnut leafminer are removed along with the pest during the cleaning of leaves from the soil as part of the sanitation. Two ingenious solutions to overcome this problem are screened-enclosures (Kehrli et al., 2005; Klungness et al., 2005), in which all infested fruits and leaves collected from the field are placed. The screen material used for its construction prevents the dispersion of the pests, but allows the escape of parasitoid wasps, thus minimizing the negative effects of crop sanitation on natural

enemies (Jang et al., 2007; Kehrli et al., 2005). The use of these devices have been shown to be highly efficient in reducing pest populations (Kehrli, 2004; Klungness et al., 2005). Damon and Valle (2002) 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 similar structures as described above should be tested in coffee plantations to harness the full biological control potential of *P. nasuta* and other parasitoids.

In addition, our two-year search for natural enemies of *H. hampei* yielded a very important discovery: a new natural enemy of *H. hampei*, and most likely the first ever recorded predator of CBB in Africa. *Karnyothrips flavipes* Jones (Thysanoptera: Phaelothripidae) was observed preying upon immature stages of *H. hampei* inside the infested berries collected from the ground.

K. flavipes is among the oldest known predatory thrips. According to Priesner (1960), the larvae and adults feed on the eggs and larvae of several species of soft scales (Coccidae), and armored scales (Diaspididae), as well as on mites and whiteflies. This is the first time *K. flavipes* has been reported being predacious on Coleopteran eggs and associated with plants different to Graminae (Steve Nakahara., pers. comm.). Field observations, laboratory trials and molecular tools have confirmed the role of *K. flavipes*, as a predator of CBB in Western Kenya. Specific primers for CBB and *K. flavipes* were designed (**Chapter 6**) which made possible the detection of CBB DNA in the DNA extractions of *K. flavipes.* Moreover, the CBB-specific primers did not produce PCR products for any of the other insects commonly found inside coffee berries (e.g., *P. nasuta*, *Aphanogmus* sp, fruitflies) except for *H. hampei* itself, indicating that *K. flavipes* is exclusively feeding on CBB life stages inside the

coffee berries. *K. flavipes* females oviposit up to 29 eggs inside an individual coffee berry, and after hatching, larvae and adults spend most of their lifetime inside the CBB galleries preying on *H. hampei*.

The CBB problem is a complex one and needs to be tackled from several angles. In addition to looking for more natural enemies of *H. hampei* and trying to understand the reasons behind the lack of success of the ones that are already being used, a sound understanding of the biology and ecology of the pest is necessary to elucidate its weakest points. In spite of the economic importance of CBB and the many years of research and dozens of publications, the bionomics of the insect are not yet fully understood. One of the reasons for this was the absence of a suitable methodology to study *H. hampei* under controlled conditions in the laboratory on its natural substrate, fresh coffee berries.

Coffee berries start to desiccate the moment they are picked from the tree. In addition, when these berries are infested by CBB, the mortality of the borers sharply increases (Benavides and Portilla, 1990). However, as stated before berries on the ground are a tremendously important source for the re-infestation of next season's coffee by *H. hampei* (Baker, 1999; Bernal et al., 1999; Bustillo et al., 1999). Bergamin (1944) and Salazar et al. (1993) observed that CBB continues to reproduce and develop in the fallen berries, and assumed that the wet soil surface slows down the desiccation process of the berries, thereby increasing the survivorship of CBB inside. We mimicked this phenomenon in the laboratory by developing an experimental set-up consisting of a mixture of plaster of Paris and charcoal that delays the desiccation of a coffee berry (Chapter 7). The buffering potential of this mixture has been successfully used in cultures of insects like thrips (Premachandra et al.,

2005), Collembola (Fox et al., 2007; Park, 2007), and mites (Muma and Denmark, 1967). In our methodology, the microclimate inside the vials kept the berries for a longer time fresh, favouring the development of CBB. This new rearing technology led to a 6-7-fold increase in survivorship of the F1 and more than 100 surviving individuals vis-à-vis 1.7 in the control. Moreover, the quality of the coffee does not influence offspring production of CBB in the novel methodology.

In conclusion, our new methodology is cheap, easy to implement when the studies are to be carried out in the vicinity of coffee plantations, and not labour intensive, thus of great practical use for any detailed study on the biology, ecology and behaviour of the insects under controlled conditions.

Previous studies on the bionomics of *H. hampei* were either carried out in the field (Borbon-Martinez, 1989; Baker et al., 1992; Ruiz, 1996), or in the laboratory with only one temperature regime (e.g., Romero and Cortina, 2004, 2007; Fernandez and Cordero, 2007). Hence we used the previously described new rearing methodology to study the effects of seven different temperature regimes on the life history of CBB. Our objective was not only to elucidate crucial life table parameters of *H. hampei*, but also to model its thermal tolerance to make inferences on the impact of climate change on the pest (Chapter 8).

Climate change is happening. An increase in the mean global temperature of 1.4° to 5.8° C is expected by the end of the 21st century (Houghton et al., 2001). Detailed studies from the temperate zones on several species report mainly positive effects on insect fitness like faster developmental rate, increased numbers of generations per year or seaon, and distribution range (Parmesan et al., 1999; Bale et al., 2002; Gomi et al., 2007; Musolin, 2007; Dingemanse and Kalkman, 2008).

However, in a recent paper Deutsch et al. (2008) predict negative effects of global warming on tropical arthropods. Surprisingly, the potential impact of global warming in tropical insects has only been studied in hematophagous insects such as malaria-transmitting *Anopheles* spp. (Diptera: Culicidae) (Patz and Olson, 2006) and the tsetse fly *Glossina pallidipes* (Diptera: Glossinidae) (Terblanche et al., 2008).

Our results on thermal tolerance of *H. hampei* confirm Stevens (1989) climatic variability hypothesis because of the demonstrated narrow range of temperatures within the beetle can thrive. The climatic variability hypothesis states that the thermal tolerance of an insect is directly proportional to the climatic variability the organism is exposed to. The extremes for CBB survival are 15 and 30°C, but development takes place only between 20 and 27°C. Although our model predicts fastest development of CBB between 27-30°C, there is clear trade-off between development time and reproductive success as previously shown for other insects (Roff, 2000).

CBB attacks and successfully develops on both *C. arabica* and *C. canephora* (Le Pelley, 1968) and is the only herbivore feeding on the endosperm of coffee due to its ability to detoxify caffeine (Vega et al., 2003b, Vega 2004). For many years, there has been controversy in the literature about the geographic origin of the pest (Bergamín, 1943; Ticheler, 1961; Benavides et al., 2005) and its original host plant(s) (Baker, 1984; Davidson, 1967) and resolving this mystery might have important implications for future breeding for host plant resistance in coffee, as well as better targeted explorations for natural enemies of CBB.

During an extensive survey, Davidson (1967) did not find CBB in Ethiopia, and Damon (2000) later speculated that the absence of the pest is due to either

specialized natural enemies, resistant varieties of *C. arabica* or exceptionally clean harvest practices in Ethiopian plantations. However, based on our estimates on the thermal tolerance of CBB it is evident that the insect cannot develop in the area around Jimma (Ethiopia), the area of origin of *C. arabica* because of the prevalent low mean annual minimum temperature there. Nevertheless, in a recent study Mendesil et al. (2004) reported widespread occurrence of CBB in South Western Ethiopia. Since we were only able to obtain meteorological data from the town of Jimma we speculate that climatic conditions in other parts of South Western Ethiopia are more conducive for the development of the insect, explaining Mendesil's et al. (2004) field observations.

According to Hodkinson (1999), distribution and range limits of an insect can be fully explained by physiological data linked to microclimatological measurements, and Campbell et al. (1974) emphasized the usefulness of the lower threshold of development and the thermal constant of an insect to elucidate its potential distribution. Similar to *C. arabica, C. canephora* is an understory tree of lowland forests. Climatological data from shaded coffee plantations in Central America (Barradas and Fanjul, 1986; Vaast et al., 2004) and East Africa (Kirkpatrick, 1935) indicate a reduction in temperature between 2-6°C depending on the region, when compared to coffee grown without shade. Considering the previous data, the annual mean temperatures of the Congo River Basin (24-26°C), and our estimates on thermal tolerance of CBB, we hypothesize that the original host plant of *H. hampei* is likely to be Robusta coffee.

How do the findings of **Chapter 8** to potential climate change effects on CBB? Data on thermal tolerance of an insect is crucial to predict the possible effects

of climate change (e.g., Hodkinson et al., 1999; Kiritani, 2007; Gomi et al., 2007; Calosi et al., 2008). The climatic data from the four locations in Colombia, Tanzania, Kenya and Ethiopia used in this study do not show an increase in temperature during the observation period. This might be due to the length of the observation period in the case of Colombia, and the poor quality of the data from East Africa. According to our estimations for the Colombian, Tanzanian and Kenyan sites (the climate data from Jimma, Ethiopia, indicated no development of CBB in this area), the potential number of CBB generations per year fluctuates between 2 and 4.5. According to our data on thermal tolerance of CBB and the calculations of warming tolerance and thermal safety margins using the formulas proposed by Deutsch et al. (2008), a small increase in temperature will lead to a considerably faster development of the pest. Since the coffee berry is a finite resource, this will lead to increased and more frequent numbers of *H. hampei* females searching for new berries to colonize a year. Such a scenario will have devastating effects in coffee growing areas like Colombia where, because of well distributed precipitation leading to multiple flowering of the plants, there is yearlong supply of coffee berries (Arcila et al., 1993). The problem will be less severe in East Africa or other coffee producing areas of the world where there is a marked and prolonged dry period, with consequent absence of berries in the field for extended periods.

Specialist herbivores do not have an adaptation capacity as great as generalist herbivores under climate change (Ward and Masters, 2007), and according to Hodkinson (1999) the eco-physiology of both insects and plants will predict the future distribution of insect pests when both host plant and herbivore are

in close synchrony. Thus, in the case of a highly specialized herbivore like *H. hampei*, the effects of climate change on the insect and the plant cannot be separated. Assad et al. (2004) for Brazil, Gay et al. (2006) for Mexico and Grid (2002) for Uganda predict that even a small increase in temperature due to climate change, will have serious consequences for coffee production in these countries, in some cases rendering production very difficult. Under a climate change scenario, species like *H. hampei*, whose distribution appears to be restricted by their host plants, will follow them as they extend their range (Hodkinson, 1999; Ward and Masters, 2007). In addition, such an expansion of its geographic host range might enable the pest also to escape some of its specialized natural enemies like parasitoids (Schönrogge et al., 1998). As recently shown in a study by Menendez et al. (2008) for the Brown Argus butterfly, *Aricia agestis* (Denis and Schiffermüller) (Lepidoptera: Lycaenidae) in the UK, specialized parasitioids may not be present in the newly colonized regions of the herbivore, or if present they might preferably attack other hosts.

Coffee is a tropical crop cultivated mainly from from 20-25°N to 24°S (Wellman, 1961). One of the strategies that insects would use to cope with climate change is shifts in their latitudinal and altitudinal distribution (Neuvonen et al., 1999; Gaston and Chown, 1999; Menendez et al., 2008). Although coffee is highly susceptible to changes in photoperiod, a latitudinal expansion in *C. arabica* and *C. canephora* is possible and our data on thermal tolerance of *H. hampei* would predict that the pest is well capable to thrive under such conditions. Moreover, an altitudinal expansion as a coping strategy in a climate change environment is potentially feasible, though there are few areas in the tropics where coffee

production could expand in altitude, e.g. the Kilimanjaro area of Tanzania, Mount Kenya, and the mountain ranges of Colombia, considering that other requirements for successful coffee production like soil type and appropriate rainfall patterns have to be met (Damatta et al., 2008). For instance, for the coffee growing area in Colombia Jaramillo (2005) estimated that an increase in temperature of 1°C would require to move the plantations by 167m in altitude to maintain the same productivity and quality in arabica coffee.

A proven strategy to alleviate the potentially negative effects of climate change on coffee production is the introduction of shade trees in coffee plantations (Lin, 2007; Damatta, 2008). Shade trees mitigate microclimatic extremes and can buffer coffee plants from microclimate variability (Beer et al., 1998), reduce high solar radiation and buffer detrimental diurnal changes in air temperature and humidity (Vaast et al., 2004; Vaast et al., 2005; Lin, 2007), leading to a decrease in temperature by up to 4°C under low altitude conditions (i.e., < 700 m.a.s.l.), and by up to 2°C under mid to high altitude conditions (i.e., > 1,100 m.a.s.l.) (Vaast et al., 2004). Moreover, Teodoro et al. (2008) recently demonstrated that densities of CBB were significantly lower in shaded versus un-shaded coffee plantations, possibly because shade coffee agro-ecosystems can serve as a refuge for beneficial arthropods (native and introduced), leading to higher levels of biological control of *H. hampei* (Perfecto et al., 1996; Tylianakis et al., 2005). Finally despite lower yields of shaded compared to un-shaded coffee, the berry weight is higher and the quality of coffee produced under shade is better (Muschler et al., 2001), with further benefits for the coffee growers such as additional income coming from timber or fruits trees or higher coffee price as result of increased quality of the product (Gordon et al., 2007).

Our results indicate that the current prospects for climate change could severely threaten coffee production, not only due to the direct effects of temperature on the plant, but also to the possibility of increased yield losses due to the expanded geographical range of *H. hampei*, the most important pest of coffee. Thus we believe the most appropriate way for coffee production systems to cope with climate change is to come back to the origins of coffee as an understory tree in the forests of Africa. In conclusion results of this study indicate the following: (i) *P. coffea* can be an important control agent of CBB when used in an augmentative manner though first efficient and economic release ratios need to be established, and the releases have to be appropriately timed to prevent superparasitism of the wasps in the field; (ii) in East Africa *P. nasuta* is clearly the most important parasitoid, but possibly its efficacy in the Americas is severely hampered by a prevalent cultural control practice; (iii) there is still scope for discovery of new natural enemies of CBB in Africa as illustrated by our results on the predatory thrips *K. flavipes*; (iv) studying the thermal tolerance of *H. hampei*, using a newly developed laboratory-based rearing methodology that mimics field conditions, allowed us to make important inferences on the impact of climate change on the potential future pest status of CBB. In general our results show that for the development of comprehensive biological control of an invasive tropical herbivore like *H. hampei* investigations need to target both the new distribution range of the pest as well as its aboriginal home.

Appendix

Table 1*. Insects that emerged from the tree and ground collected coffee berries in a study aiming at identifying new natural enemies of the coffee berry borer (chapter 4).

Species	Origin	Habit
HYMENOPTERA		
Aganoidae		
Pleistodontes sp	Ground	Fig wasp
Aphelinidae		
Coccophagus sp	Tree	Parasitoid of mealybugs
Bethylidae		
Prorops nasuta	Ground - tree	Primary CBB parasitoid
Cephalonomia stephanoderis	Tree	Primary CBB parasitoid
Goniozus sp	Ground	Possible CBB parasitoid
Braconidae		
Psyttalia sp	Tree	Fruit fly parasitoid
Fopius sp. Poss.undescribed	Tree	Fruit fly parasitoid
Asobara sp.	Ground	Fruit fly parasitoid
Pauesia sp	Ground	Fruit fly parasitoid
Ceraphronidae		
Aphanogmus goniozi	Ground	Hyperparasitoid of *P. nasuta*
Eulophidae		
Nesolyxn sp	Ground	Parasitoid of Lepidoptera
Phymastichus coffea	Ground - tree	
Encyrtidae		
Non identifiable		
Copidosoma sp	Ground	Polyembrionic parasitoid of Lepidoptera
Eurytomidae		
Philolema sp	Tree -ground	
Figitidae		
Ganaspis sp	Tree	Fruitfly parasitoid
Formicidae		

Tapinoma sp	Ground	Possible CBB predator
Myrmicidae		
Not identifiable to genus	Ground	
Ichneumonidae		
Cryptinae	Ground	
Platigasteridae		
Allotropa sp	Ground	Fruitfly parasitoid
Fidiobia sp	Tree	
Pteromalidae		
Pachycrepoideus nr. *schedli*	Ground	Primary parasitoid of CBB
Oxsychus sp	Ground	Known from stemborer curculionidae
Scelionidae		
Trissolcus sp	Ground	Scelionidae:
Torymidae		
Torymoides sp	Tree	Associated with cecidomyiid galls
DIPTERA		
Chloropidae	Ground	Scavenger
COLEOPTERA		
Sophronica sp.	Ground	Pest of coffee
Cryptolestes sp	Ground	Possible predator of CBB:
Staphylinidae	Ground	Possible predator of CBB:
Nesolyxn sp		
THYSANOPTERA		
Karyothrips flavipes	Ground	Predator of CBB

* All taxonomic identifications presented in this table were carried out in the Systematic Entomology laboratory of the United States Department of Agriculture (USDA), Beltsville, Maryland, by Drs. Matthew Buffington and Michael Gates.

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Declaration by candidate

I, Juliana Jaramillo Salazar, declare that this thesis, entitled "Biology, Ecology and Biological Control of the Coffee Berry Borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae)" is an original piece of work conducted by myself and has not been submitted for a degree in any other University.

Hannover, October 2008

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