UNIVERSIDADE FEDERAL DE VIÇOSA

JÉSSICA MAYARA COFFLER BOTTI

ROLE OF GREEN LACEWINGS AND ANTS ON COFFEE BERRY BORER PREDATION

VIÇOSA - MINAS GERAIS
2021

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Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

Orientadora: Madelaine Venzon

Coorientadores: Gustavo Júnior de Araújo
Maria Augusta Lima Siqueira

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Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

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Botti, Jéssica Mayara Coffler, 1992-

B751r
2021

Role of green lacewings and ants on coffee berry borer predation / Jéssica Mayara Coffler Botti. – Viçosa, MG, 2021.
85 f. : il. (algumas color.) ; 29 cm.

Orientador: Madelaine Venzon.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Café - Doenças e pragas - Controle biológico. 2. *Hypothenemus hampei*. 3. Insetos predadores. 4. Formigas. 5. Crisopídeo. I. Universidade Federal de Viçosa. Departamento de Entomologia. Programa de Pós-Graduação em Entomologia. II. Título.

CDD 22. ed. 633.7397

Bibliotecário(a) responsável: Renata de Fatima Alves CRB6/2578

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APROVADA: 30 de julho de 2021.

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A Deus, por ser essencial em minha vida e autor do meu destino.

Aos meus queridos pais, Margareth Maria Coffler e Antonio Carlos Botti, a minha irmã Tálita Coffler Botti Braz e ao meu namorado Natan Venturini Rizzi pelo amor, apoio e incentivo a mim ofertado ao longo da minha caminhada, nos momentos alegres e difíceis.

DEDICO

AGRADECIMENTOS

À Universidade Federal de Viçosa pela oportunidade da realização destes estudos.

À Empresa de Pesquisa Agropecuária de Minas Gerais -EPAMIG- Unidade Regional Zona da Mata e Fazenda Experimental de Patrocínio, pela estrutura oferecida para a realização deste trabalho.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico pela concessão da bolsa de estudos.

À Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café (CBP & D-Café) pelo apoio na realização do projeto.

À minha orientadora Madelaine Venzon, por ter me dado a oportunidade de realizar um trabalho do qual me orgulho, pela orientação e apoio imensurável para a realização deste trabalho.

Aos meus coorientadores Maria Augusta e Gustavo, pela ajuda nas análises estatísticas e ajuda em todos os momentos.

Ao membros da banca Dra Mariella, Dra Maria Augusta, Dr Angelo e Dr Tito por terem concordado em participar da banca de avaliação desta tese e em contribuir para sua melhoria.

À meu colega Júlio pela ajuda na identificação das formigas.

Ao laboratório de Toxicologia em especial ao Professor Raul e ao colega Lírio por disponibilizarem a utilização do raio-X.

Ao laboratório de Acarologia em especial ao Professor Angelo e a colega Vanessa por disponibilizarem o uso de equipamentos para realização de fotos e vídeos.

Ao funcionário Mario do viveiro de café por permitir a coleta de frutos de café para a realização dos experimentos.

Aos colegas de laboratório (atuais/contemporâneos/agregados) Elem, Mayara, Juliana Martinez, Juliana Maria, Gabriel, Fernanda, Jéssica, Tércio, Thais, Caro, Alvaro, João, Jessily, Gabriel Pio, Mathias, André, Pedro por toda ajuda.

A todos os colegas da Epamig de Viçosa, pelos anos de convivência, em especial ao Miguel por todo apoio nas coletas em campo.

A todos os colegas da Epamig de Patrocínio, pelo suporte nos experimentos de campo, Jaime, Diego, Greice, Nenzin, Juliana, Marcela, safristas e todos os funcionários que me apoiaram nos experimentos.

A Jéssica uma amiga de republica que amo tanto.

A Mayara e Elem pela amizade que construímos durante esses anos. Por não medirem esforços para construírem essa Tese comigo. Por tudo que passamos juntas, viagens, medos e muitas conquistas que ficarão eternamente na minha memória. Foram muitas risadas, sempre juntas. Já estou com saudade “não se vá, não me abandone por favor...”.

A toda minha família pelo apoio, torcida e confiança que sempre depositam em mim; pelos momentos que não estivemos juntos e souberam entender. Em especial a minha pequena Luísa que contava os dias para me ver.

Ao meu eterno namorado, amigo e companheiro de todas as horas Natan, pela compreensão, carinho, companheirismo e apoio quando mais precisei, inclusive nas coletas de campo. Como você mesmo disse “entramos nessa juntos, para sairmos juntos”. Te amo!

A minha irmã Tálita e sua família Wanderson e nosso pequeno Samuel, por estarem sempre ao meu lado me ajudando, pela incessante demonstração de carinho e companheirismo.

Aos amores da minha vida, minha mãe Margareth e meu pai Antonio, pelos ensinamentos diários e pelo apoio em todos os momentos. Eu amo vocês!

A Deus, por ter me dado coragem e força para vencer os obstáculos dessa longa caminhada e por não ter me abandonado se quer por um instante!

Nossos sonhos de

vencer temos que juntar.

Só essa paixão no

coração nos guiará.

(Eiichiro Oda)

RESUMO

BOTTI, Jéssica Mayara Coffler, D.Sc., Universidade Federal de Viçosa, julho de 2021. **O papel de crisopídeos e formigas na predação da broca-do-café**. Orientadora: Madelaine Venzon. Coorientadores: Maria Augusta Lima Siqueira e Gustavo Júnior de Araújo.

A broca-do-café *Hypothenemus hampei* é a praga mais severa da cultura do café no mundo. Suas larvas abrem galerias dentro do endosperma dos frutos de café, causando perdas significativas na produtividade e na qualidade dos grãos. Além disso, o controle desta praga é dificultado devido ao seu hábito críptico de viver dentro dos grãos. A redução de fontes de abrigos e de alimentos alternativos para insetos predadores que realizam o controle biológico da broca-do-café, ocorre devido à simplificação da paisagem associada aos cultivos convencionais de café. Portanto, se faz necessário o uso de medidas que visam contornar esses problemas causados pela implantação de cultivos convencionais, a fim de aumentar e manter os inimigos naturais da broca-do-café nessas áreas. Dentre essas medidas, diversificação da paisagem, através do consórcio estratégico com plantas que possam fornecer recursos alimentares e abrigo aos inimigos naturais. Neste trabalho foi implementado uma diversificação estratégica em cultivos de café, sem utilização de pesticidas, consorciando a *Inga edulis*, *Varronia currasavica*, *Senna macranthera* e plantas espontâneas, reconhecidas por favorecer o controle biológico da broca-do-café através da manutenção de formigas predadoras na área. Durante as avaliações em campo, foi encontrado um adulto da família Chrysopidae que emergiu de um fruto brocado coletado na área de cultivo de café diversificado. No capítulo I foi comprovada a hipótese de que larvas nuas do crisopídeo *Chrysoperla externa* são capazes de entrar em frutos de café brocados e remover ovos e larvas da broca-do-café dos frutos, mostrando-se eficiente no controle da praga. No capítulo II foi testado se, o comportamento de transportar detritos no dorso reduz o acesso às galerias da broca-do-café por larvas lixeiras de *Ceraeochrysa cubana* em relação a *C. externa*. Os resultados mostraram que as larvas de primeiro instar das duas espécies (*C. externa* e *C. cubana*) são capazes de entrar nas galerias da broca-do-café e sobreviver mais na presença de frutos brocados do que na presença de frutos sadios e sem alimento. Porém, *C. cubana* se mostrou menos eficiente em entrar nas galerias, mostrando que o lixo pode limitar a predação da broca-do-café dentro do fruto. As larvas de terceiro instar de *C. externa* predaram adultos da broca-do-café e diminuíram em 10% a capacidade da broca em infestar os frutos, o que mostra que *C. externa* pode ser mais eficiente no controle da broca-do-café devido a sua facilidade de acessar as galerias. Já no terceiro capítulo foi avaliada a riqueza e abundância de formigas predadoras da broca-do-café, a taxa de infestação da broca-do-café e a produção do café no sistema diversificado em comparação ao sistema convencional de café. Os resultados mostraram que a diversificação estratégica aumentou a riqueza e abundância de formigas predadoras, diminuiu a infestação da broca-do-café e não afetou a produção do café. Portanto, a diversificação estratégica com *I. edulis*, *V. currasavica*, *S. macranthera* e plantas espontâneas aumentou o controle natural da broca-do-café por formigas predadoras e crisopídeos, além de não ter reduzido a produção do café.

Palavras-chave: *Hypothenemus hampei*. Controle biológico conservativo. Chrysopidae**.**

ABSTRACT

BOTTI, Jéssica Mayara Coffler, D.Sc., Universidade Federal de Viçosa, July, 2021. **Role of green lacewings and ants on coffee berry borer predation**. Adviser: Madelaine Venzon. Co-advisers: Maria Augusta Lima Siqueira and Gustavo Júnior de Araújo.

The coffee berry borer (CBB) *Hypothenemus hampei* is the most severe pest of the coffee crop in the world. Larvae open galleries inside the endosperm of coffee fruits, causing significant losses in yield and fruits quality. In addition, control of this pest is hampered due to its cryptic habit of living inside the fruits. The reduction the sources of shelter and alternative food for predatory insects that carry out the biological control of CBB is due to the simplification of the landscape associated with conventional coffee crops. Therefore, it is necessary to use measures that aim to decrease these problems caused by the implantation of conventional crops, in order to increase and maintain the CBB natural enemies in these areas. Among these measures, landscape diversification, through strategic diversification with plants that can provide food resources and shelter to natural enemies. In this work, a strategic diversification in coffee crops was implemented, without the use of pesticides, associated with *Inga edulis, Varronia currasavica*, *Senna macranthera* and non-crop plants, recognized for favoring the biological control of CBB through the maintenance of predatory ants in the area. During field evaluations, an adult of the Chrysopidae family was found that emerged from a coffee bored berries collected in the diversified coffee crop area. In Chapter I, I confirmed the hypothesis that naked larvae the of *Chrysoperla externa* are able to enter the galleries and remove CBB eggs and larvae from inside the fruits, proving to be efficient in pest control. In Chapter II, I tested whether the behavior of transporting debris on the back reduces the access to the CBB galleries by trash-carry larvae of *Ceraeochrysa cubana* in relation to *C. externa*. The results showed that the first instar larvae of the two species (*C. externa* and *C. cubana*) are able to enter the CBB galleries and survive longer in the presence of coffee bored berries than in the presence of healthy berries and without food. However, *C. cubana* was less efficient in entering the galleries, showing that the trash can limit the predation of CBB inside the fruit. Third instar larvae of *C. externa* preyed on CBB adults and reduced the capacity of CBB to infest the fruits by 10%, which shows that *C. externa* can be more efficient in controlling the CBB due to its ease of accessing the galleries. In the Chapter III I evaluated the richness and abundance of predatory ants of CBB, the rate of infestation of CBB and the coffee yield in the diversified system implemented, comparing it to the conventional coffee system. The results showed that strategic diversification increased predator ant richness and abundance, decreased CBB infestation and did not affect coffee yield. Therefore, strategic diversification with *I. edulis*, *V. currasavica*, *S. macranthera* and non-crop plants increases the natural control of CBB by predatory ants and green lacewings, in addition to not reducing coffee yield.

Keywords: *Hypothenemus hampei*. Conservative biological control. Chrysopidae**.**

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GENERAL INTRODUCTION

Brazil is the world's largest producer (3,009,402 ton) and exporter (2,230,872 ton) of coffee (FAOSTAT 2019). Besides this expressive role of the crop for the country, several factors can negatively impact the coffee production and, among them the attack by several arthropod pests. One of the key pest in the crop is, the coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) that is, native to Central Africa and, is the most damaging insect pest of coffee worldwide (Le Pelley 1968; Damon 2000; Vega et al. 2009; Cure et al. 2020). It is the only pest able to feed and complete the life cycle in coffee fruits, causing losses in yield and quality (Damon 2000; Jaramillo et al. 2006; Vega et al. 2009; 2015). CBB adult females infest the coffee berry when the moisture content is less than 80%, approximately 120-150 days after flowering (Laurentino and Costa 2004). Females pierce the fruit, making a small entrance hole (0.6–0.8 mm wide), usually close to the floral disc, and deposit their eggs into the endosperm (Damon 2020; Vega et al. 2009, 2015). Females can oviposit over 100 eggs during their oviposition period of 11 to 40 days (Jaramillo et al. 2009). After the eggs hatch, the larvae feed on the endosperm and establish a symbiosis with bacteria that allows the degradation of the caffeine contained in the seeds. The mode of transmitting caffeine-degrading bacteria is unknown (Ceja-Navarro et al. 2015; Vega et al. 2021). In Brazil, the losses caused by this insect are estimated from US$215 up to 358 million annually (Oliveira et al. 2013). Due to its cryptic habit of living inside the fruit, the control of CBB is difficult. Thus, integrative measures are key to deal with this pest, being necessary to use cultural, behavioral and biological control (Damon 2000; Vega et al. 2009; Infante 2018; Johnson et al. 2020).

The biological control of CBB involves entomopathogenic fungi, parasitoid and predators (Damon 2000; Vega et al. 2009; Infante 2018; Jonson et al. 2020). The most used fungus is *Beauveria bassiana* (Balsamo) Vuillemin, spraying it in the crop as a curative control measure. The greatest difficulty in using the fungus is that it is highly dependent on climate conditions, resulting in a variation in CBB mortality (González et al. 1993, Bustillo 2006; Greco et al. 2018; Hollingsworth et al. 2020). Additionally, the entomopathogenic fungi act on contact (Alves 1998), which is made difficult because the CCB lives inside the fruits. Parasitoid wasps have shown different results in CBB mortality (Damon 2000; Aristizábal et al. 2016), as their establishment depends on both the environment (Infante et al. 2001; Vega et al. 2015; Johnson et al. 2020) and on their mass rearing. The CBB has many predators such as Hymenoptera (Formicidae) (Armbrecht and Gallego 2007; Larsen and Philpott 2010; Gonthier et al. 2013; Morris and Perfecto 2018), Thysanoptera (Phlaeothripidae) (Jaramillo et al. 2010; Rezende et al. 2014; Pantoja 2019), Hemiptera (Anthocoridae) (Bustillo et al. 2002) and Coleoptera (Silvanidae, Laemophloeidae, Cucujidae) (Vega et al. 1999; Bustillo et al. 2002; Follett et al. 2016; Sim et al. 2016). Ants are the most studied predators, and they are able to feed on different phases of the CBB, inside and outside the coffee fruit. Their conservation in coffee fields improves CBB control (Morris and Perfecto 2018), but they can be aggressive and negatively seen during harvest by farm workers (Philpott and Armbrecht 2006; Offenberg 2015).

The biological control of CBB can be increased through attraction and permanence of its natural enemies in the crop. In order to attract and maintain natural enemies in coffee crops, one strategy is the association with other plant species (Amaral et al. 2013; Rezende et al. 2014, 2021; Rosado et al. 2021). The choice of plants must be strategic in order to benefit only the beneficial insects (Meyer et al. 2009), by providing resources such as alternative food (pollen and nectar), shelter and refuge (Rezende et al. 2014). In addition, these plants should not host the key pests of the target crop, not compete for water and nutrients with the coffee plants and, finally should not need laborious cultivation as several pruning, fertilization, and constant irrigation (Venzon et al. 2006; Lavandero et al. 2006; Venzon and Sujii 2009; Souza et al. 2010; Venzon et al. 2011).

In this thesis, I investigated whether strategic vegetable diversification in *Coffea arabica* crops favors the biological control of CBB by predators. I used as a model for strategic diversification the *Inga edulis*, *Varronia currasavica*, *Senna macranthera* and non-crop plants. These plants chosen can provide food, such as nectar and pollen, for several natural enemies through extrafloral nectary (*I. edulis* and *S. macranthera*) and/or present constant blooms (*V. curassavica*). During field evaluations, I found a Chrysopidae larvae in bored berries. Therefore, I performed laboratory experiments to assess whether this predator is a potential CBB biological control agent (Chapter I and II). I also investigate whether strategic vegetation diversification in coffee crops increases biological control of CBB by predatory ants. For this, I compared the predation rate, abundance and richness of predatory ants and CBB infestation rate between diversified and conventional monoculture coffee systems (Chapter III).

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Neotropical Entomology
https://doi.org/10.1007/s13744-021-00884-0

SCIENTIFIC NOTE

Predation of Coffee Berry Borer by a Green Lacewing

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Received: 14 April 2021 / Accepted: 17 May 2021

Abstract
We report here for the first time, the predation of coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari) by a green lacewing species, *Chrysoperla externa* (Hagen). We showed in laboratory the predator ability to access CBB galleries, remove pest immature stages, and prey on them. We also observed predation by third instar larvae on CBB adults. With this note, we add a new predator to the reported list of species still little explored of CBB control.

Keywords Biological control · *Hypothenemus hampei* · *Chrysoperla externa* · *Coffea* spp

Native from Central Africa, the coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae, Scolytinae) is considered to be the most damaging pest of coffee worldwide (Le Pelley 1968; Damon 2000; Vega et al. 2009; Cure et al. 2020). It is a cosmopolitan pest that currently exists in all coffee producer countries except in Australia, and Nepal (Johnson et al. 2020). Coffee berry borer is also considered one of the most important biological invasions of tropical agroecosystems, occurred in Brazil (Infante et al. 2014). It is the only species that can feed and complete its cycle on coffee seeds, due to the presence of bacterial symbionts in the gut that degrade caffeine (Ceja-Navarro et al. 2015; Vega et al. 2021). Females of CBB bore into the berries and oviposit inside the coffee berry endosperm. Hatched larvae feed on the seeds resulting in losses of quality and quantity of the marketable coffee (Damon 2000; Jaramillo et al. 2006; Vega et al. 2009).

Successful control of CBB is rather difficult due to its crypt life history. Females require 2–8 h to enter the berry (Wrigley 1988; Mendesil et al. 2004) leaving a short window to control the exposed adult stage. Despite the overuse of synthetic pesticides, CBB continues to cause major economic losses in coffee crops (Oliveira et al. 2013; Infante et al. 2014; Johnson et al. 2020). Integrative measures are thus key to deal with this pest, and cultural, behavioral, and biological control approaches are needed (Damon 2000; Vega et al. 2009; Infante 2018; Johnson et al. 2020). Spraying entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin is one of the widespread curative biological control measures, as the formulated product is commercially available in most coffee producer countries (Mascarin and Jaronski 2016). However, its efficiency is highly dependent on climatic conditions and CBB adult mortality is extremally variable (González et al. 1993; Bustillo 2006; Greco et al. 2018; Hollingsworth et al. 2020). Releases of parasitoid wasp species showed variable action on CBB populations (Damon 2000; Aristizábal et al. 2016), due to their lack of establishment in new world coffee and challenges in their mass rearing (Infante et al. 2001; Vega et al. 2009, 2015; Johnson et al. 2020)

Predators are the least studied natural enemies of CBB, except for ants (Armbrecht and Gallego 2007; Larsen and Philpott 2010; Gonthier et al. 2013; Morris et al. 2018). Ants (e.g., *Pheidole* spp., *Azteca* spp., and *Solenopsis* spp.) can prevent CBB damage by impeding the borrowing activity of CBB females, by removing CBB from within-fruit tunnels as well as by feeding on CBB immatures. The conservation of these predators in coffee fields encourages CBB control

Edited by Lessando Moreira Gontijo

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Published online: 13 July 2021

Fig. 1 First instar larvae of *Chrysoperla externa* entering in a CBB gallery in a green coffee berry

(Morris et al. 2018), but coffee farm workers typically have a negative view of ants due to their aggressiveness during harvesting (Philpott and Armbrecht 2006; Offenberg 2015). Other reported predators of CBB are species from Thysanoptera (Phlaeothripidae) (Jaramillo et al. 2010; Rezende et al. 2014), Hemiptera (Anthocoridae) (Bustillo et al. 2002), and Coleoptera (Silvanidae, Laemophloeidae, Cucujidae) (Vega et al. 1999; Bustillo et al. 2002; Follett et al. 2016; Sim et al. 2016).

To our knowledge, green lacewings (Neuroptera: Chrysopidae) were never reported as predators of CBB. Here, we report and confirm the predator accessibility to CBB galleries and feeding on immatures. During a survey in coffee plots located in Patrocínio, state of Minas Gerais, where coffee plants are associated to *Inga edulis* Mart. (Fabaceae), *Senna macranthera* (Collad.) Irwin et Barn. (Fabaceae), *Varronia curassavica* Jacq. (Cordiaceae), and spontaneous plants, we collected bored fruits to evaluate the presence of natural enemies. We collected ripe bored berries, individualized them in the field, in plastic vials (3.5 cm high and 2.0 cm of diameter), and we brought them to the Laboratory of Entomology at Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG). The individualized ripe bored berries were kept under controlled conditions (25 ± 2°C, 12-h photoperiod, RU 70 ± 2) during 1 month. After that, during the process of dissecting the bored berries, we found a green lacewing adult inside one of the vials. As all berries were fully examined before individualization and no pupae outside the bored berries were found, we raised the question whether Chrysopidae would access and feed on CBB.

Thus, we test the hypothesis that Chrysopidae larvae are able to enter into CBB galleries, remove immatures, and feed on them. For testing it, we evaluated whether the green lacewing *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae), a species commonly found in coffee crops (Ribeiro et al. 2014; Martins et al. 2019), has this behavior. Larvae of *C. externa* were taken from a laboratory rearing at EPAMIG, started with insects originated from AMIPA (Associação Mineira dos Produtores de Algodão). The taxonomic identification of *C. externa* from AMIPA rearing was done by Isadora Peres and Prof. Luís Cláudio Paterno Silveira (Federal University of Lavras). We offered to the first instar larvae of *C. externa*, bored coffee berries with CBB immatures in three different maturation stages: green, ripe, and dry.

The experiment was carried out in arenas made with a Petri dish (1.0 cm high and 3.5 cm of diameter) containing a bored berry and one first instar larvae of *C. externa*. The green lacewing was observed for 30 min to evaluate whether it enters or not in the bored berry. After observations, we open the berries with the aid of a scalpel to confirm the presence of CBB immatures. Berries with CBB immature absence were excluded from the analysis. For each maturation stages, we did 30 replicates. The green lacewing entered in the gallery of 53% of the green bored berries (Fig. 1 and Video 1), in 60% of the red

Fig. 2 First instar larvae of *Chrysoperla externa* preying on CBB egg removed from a green coffee berry (**a**, **b**)

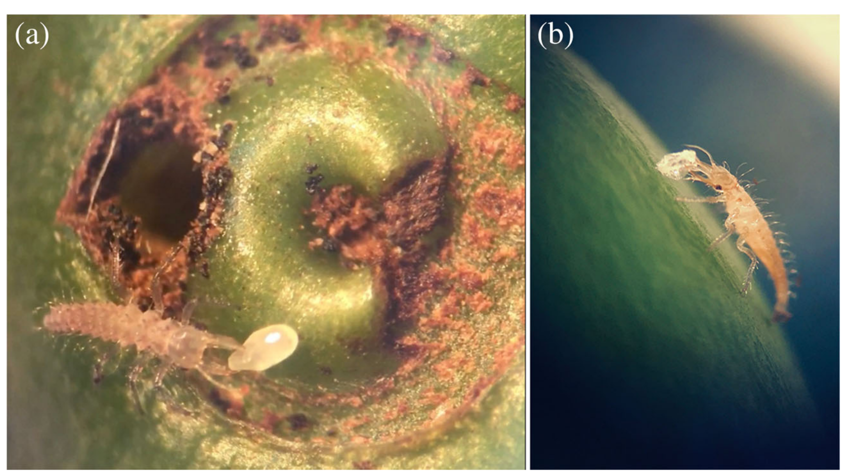

Fig. 3 First instar larvae of *Chrysoperla externa* preying on CBB larvae removed from a green coffee berry

bored berries and in 46% of the dry bored berries. During the evaluation, we observed that *C. externa* larvae removed and preyed on CBB eggs (Fig. 2 and Video 2) and on larvae (Fig. 3). Therefore, our results show the ability of a green lacewing species to prey on CBB. Additionally, to this experiment, we observed that larger larvae of *C. externa*, third instar, are able to prey on CBB adults (Fig. 4 and Video 3). Studies involving the nutritional value of the CBB for Chrysopidae species and predation rate are in progress.

Knowledge about the complex of predators associated with CBB and the strategies to conserve them in coffee crops will compose the bunch of measures to manage such important coffee pest. Conservation of Chrysopidae in coffee crops can be achieved by using cover crops, by associating extrafloral possessing trees and by maintaining spontaneous plants (Venzon et al. 2006; Rosado 2007; Rezende et al. 2014; Venzon et al. 2019). These associated plants provide important food resource for Chrysopidae, as pollen and nectar (floral and extrafloral), and refuge from intraguild predation. Non-prey food is essential for *C. externa* adults and may also be used by larvae to supplement or complement their diet during prey scarcity on in the presence of inferior prey (Venzon et al. 2006; Oliveira et al. 2010). When a curative measure has to be applied, releases of *C. externa* would be possible, as the species is commercially available in Brazil. The combined strategies are under study and our aim is to provide technical information about conservation and augmentation of *C. externa* in coffee crops for a sustainable CBB control.

Fig. 4 Third instar larvae of *Chrysoperla externa* preying on CBB adult

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13744-021-00884-0.

Acknowledgements We thank AMIPA for providing *C. externa* eggs for our laboratory rearing for the experiment.

Author contribution JMCB: methodology, investigation, writing—original draft; EFM: methodology; writing; MLF: methodology, writing; MV: supervision, funding acquisition, writing—review and editing.

Funding We thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café (CBP D-Café) for research grants and fellowships to the authors.

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Chapter II[1]

Predation of coffee berry borer by Chrysopidae species: when trash matters

Abstract:

BACKGROUND: The cryptic life history of the coffee berry borer (CBB) inside coffee berries become hard the control of this pest. Integrative measures such as cultural, behavioral and biological control are necessary to successfully control of CCB. Different taxonomic groups carry out the biological control of CBB. We evaluated the predation of CBB by one naked (*Chrysoperla externa*) and one trash-carry larvae (*Ceraeochrysa cubana*) green lacewing species.

RESULTS: We found that first instar larvae (*C. externa* and *C. cubana*) entered in the galleries of CBB. The first instar larvae of green lacewing (*C. externa* and *C. cubana*) are capable of preyed CBB eggs, larvae and pupae offered out of the berries. CBB adults were preyed only by third instar larvae of both species of green lacewing. Besides that, *C. externa* had a greater potential to survive in the presence of bored berries. Also third instar larvae limited the colonization of CBB in coffee berries.

CONCLUSION: Green lacewing larvae (*C. externa* and *C. cubana*) are predators of CBB. Effective strategies to manage CBB can be made through understandings interactions among prey and natural enemies.

Keywords: *Hypothenemus hampei*, *Chrysoperla externa*, *Ceraeochrysa cubana*, *Coffee* spp.

[1] Chapter formatted in the norms of the Journal Pest Management Science.

1 Introduction

Green lacewing are predators of Chrysopidae family (Neuroptera) with more than 1400 species distributed in 82 genera (Oswald 2007). Adults have green colors, large membranous wings with a characteristic modified venation (Brooks and Barnard 1990), and varied feeding habits (carnivorous, glyciphagous and polliniphagous) (Principi and Canard 1984). The larvae are campodeiform and can be 'naked' or trash-carrying (Souza and Bezerra 2019). The trash-carrying larvae carries debris on the dorsum, such as prey exoskeletons, exuviae, small pieces of dried leaves or wood, sand or soil, for physical protection and camouflage (Canard and Duelli 1984; Tauber et al. 2000, 2014). Naked larvae do not carry any debris on the dorsum and uses other protective strategies, such as high mobility, agility and production of repellent secretions (Canard and Duelli 1984; Haruyama et al. 2012; Souza and Bezerra 2019).

Some green lacewing species are considered suitable biological control agents due to their high voracity and foraging capacity, wide geographical distribution, compatibility with several agricultural ecosystems, pesticide resistance and easy mass rearing under laboratory conditions (Canard and Principi 1984; Godoy et al. 2010; Souza and Bezerra 2019). They are often found in coffee crops (Pappas et al. 2011; Ribeiro et al. 2014; Barbosa et al. 2019; Martins et al. 2019; Martins et al. 2021; Botti et al. 2021), but their role in biological control of them requires more study as their relationship with coffee pests in field is little understudied.

Coffee is attacked by different pests (Pereira et al. 2007; Carvalho et al. 2019; Avelino et al. 2012; Johnson et al. 2020). The coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari 1867) (Coleoptera: Curculionidae, Scolytinae) is one of its the major coffee pests (Le Pelley 1968; Damon 2000; Vega et al. 2009; Cure et al. 2020). Females of this pest oviposit into berries and their offspring develops feeding on their endosperm, which negatively affects the crop causing significant losses in yield and quality of berries (Damon 2000; Jaramillo et al. 2006;

Vega et al. 2009). Besides that, the cryptic nature of CCB (i.e., life cycle inside the coffee berry) difficult its control. Therefore, it is necessary to use integrative management strategies, such as cultural, behavioral and biological control (Damon 2000; Vega et al. 2009; Infante 2018; Johnson et al. 2020). The biological control involves the use of entomatogenous fungi, parasitoids and predators, but the use of these agents is difficult because they require favorable environmental conditions and are difficult to mass-reared to be released in coffee crops. The knowledge easy mass-readed natural enemies can allows a new approach to be apply on biological control of the CBB.

Green lacewing can be mass-reared and commercially available for release as either eggs or larvae (Souza and Bezerra 2019). During our field evaluations, we found a Chrysopidae adult emerging from a coffee bored berries. We further investigated the behaviors of *Chrysoperla externa*, that has naked larvae, and we observed it removing CBB eggs and larvae from inside the coffee berries, thus confirming CBB predation (Botti et al. 2021, Chapter 1). Given this fact, we investigated whether other species of the Chrysopidae family are efficient CBB predation and whether trash-carrying can limit access to the CBB galleries, which is limited to a 1.0 mm hole (Baker et al. 2002; Alba-Alejandre et al. 2018). The presence of debris on Chrysopidae larvae dorsum increase larvae size compared to naked larvae. Here, we investigated CBB predation by two green lacewing species, one with 'naked' larvae (*C. externa*) and one with trash-carrying larvae (*Ceraeochrysa cubana*). We start by addressing the follow question: Does CBB gallery access differ between naked and trash carrying Chrysopidae larvae? Having found that both are able to enter into CBB galleries, we evaluated Chrysopidae predation rate and survival on CBB prey. Finally, we investigate whether Chrysopidae larvae prevent the CBB adult bur from perforating the berries.

2 Materials and Methods

2.1 Rearing of green lacewing

We used green lacewing larvae (*C. externa* and *C. cubana*) from the rearing of the Laboratory Entomology at Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG). Both predator species were reared following the methodology of Venzon et al. (2006). Adults were kept in PVC tubes cages (15x15 cm) lined with paper towels. The cages were supported on plastic trays lined with paper towels and closed with PVC film. They are fed with a diet of yeast and honey diet (1:1) offered on a parafilm stripe hanged inside the cage. The water was provided in a glass bottle (10 mL) with cotton soaked. Diet and water were replaced twice a week. Paper towels and PVC film where females lay their eggs were replaced weekly. Eggs and larvae were individualized in plastic pots (10.0 cm high and 4.0 cm of diameter). The larvae were fed with eggs of *Anagasta kuehniella* (Lepidoptera: Pyralidae), added every three days, until pupation. The rearing unit are kept at 25 ± 2°C, 70 ± 10% RH and 12h photophase.

2.2 Access of Chrysopidae larvae to CBB gallery

In order to evaluate the potential of *C. externa* and *C. cubana* as CBB predators, we evaluated their ability to get into the bored berries. We collected coffee bored berries in three different maturation stages (green, ripe and dry), in coffee crops at Federal University of Viçosa, Viçosa, Minas Gerais. In the laboratory, we set up arenas using Petri dishes (1.0 cm high and 3.5 cm of diameter) containing bored berries and we placed one berry bored of each maturation stage in each arena. Then, we released one green lacewing (first or second instar) and observed for 30 minutes whether it enter into the gallery. For each maturation stages and each species, were carried out 30 replicates.

2.3 CBB predation by *Chrysoperla externa* and by *Ceraeochrysa cubana*

Both green lacewing species were able to enter in the bored berries, thus we conducted an experiment to evaluate their ability to prey on different CBB stages. We removed eggs, larvae, pupae and adults of CBB from bored berries. We offered to the first instar larvae of *C. externa* or *C. cubana* (instar that most entered in the bored berries) an individual prey of each stage, in plastic pots (3.5 cm high and 2.0 cm of diameter), and we closed it with PVC film. We also tested CBB adult female predation by third instar larvae of *C. externa* and *C. cubana*, because we observed these larvae attacking and preying on CBB adult in other experiments. Besides that, third instar larvae of *C. externa* and *C. cubana* do not have access the CBB gallery that prevents predation of CBB eggs, larvae and pupae. Each treatment (prey stage) was replicated 30 times for each species. The pots were maintained in the Laboratory Entomology of EPAMIG and under 25 ± 2 ° C and 12-hour photoperiod. After 24 hours, we evaluated the predation, considering wounded or dead individuals.

2.4 *Chrysoperla externa* and *Ceraeochrysa cubana* survival on bored berries

This experiment was conducted to investigate whether green lacewing larvae (*C. externa* and *C. cubana*) can survive by feeding on CBB inside the berries. First, we verified the presence of CBB immature phases and the position of the CBB adult in bored berries in a Faxitron model LX-60 cabinet X-ray system at the Insecticide Ecotoxicology Laboratory at UFV (Fig. 1). Bored berries were exposed for 20 seconds to the x-ray with a voltage of 36 Kilovoltages (Pantoja 2018). We used green bored berries, as it is the maturation stage that CBB starts its infestation. We did not use ripe berries because they would mold and interfere with the survival of the green lacewing. Dry berries were not used because on the X-ray it is not possible to detect all the CBB galleries. Females of CBB can impede predator access to their immatures by blocking gallery opening with their body (Pantoja 2018). Thus, survival of the two Chrysopidae species was evaluate on the four treatments: (I) green bored berries without CBB adult at the entrance of the gallery; (II) Intact green berries (without CBB); (III) *A. kuehniella* eggs (positive control); and (IV) absence of food (negative control). Each treatment was placed in plastic pots (3.5 cm high and 2.0 cm of diameter) with one first instar larva of the green lacewing (*C. externa* and *C. cubana*). Each treatment was replicated 30 times for both predator species. The food was not replaced during the evaluations and survival was assessed daily, until the green lacewing death.

2.5 CBB infestation in the presence of *Chrysoperla externa* and *Ceraeochrysa cubana*

Here, we tested whether the green lacewing is able to prevent CBB of infesting the berries. We used green bored berries collected in coffee crops at UFV. In the laboratory, we set up arenas using a transparent Gerbox (11 cm long x 11 cm wide x 3.5 cm high) covered with PVC film, containing a thin wooden rod (11 cm long x 3 mm in diameter) and five green coffee berries without CBB infestation fixed with hot glue on it, mimicking a coffee rosette (Fig. 2). We transferred two adult females of the CBB and one third instar green lacewing larva to each arena. The experiment consisted of two treatments, the presence and the absence of green lacewing larvae. We did 30 repetitions for each treatment and each species. According to Mendesil et al. (2004), the perforation of the CBB in coffee berries between 5-8 hours after its contact with the berries. In order to estimate the interference of green lacewing on CBB infestation capacity, the number of bored berries was evaluated after 24 hours from predator introduction. We also count the number of CBB dead.

2.6 Statistical analysis

We used generalized linear models (GLMs) with binomial error distribution to evaluate the success of green lacewing in accessing the CBB galleries at different maturation stages. The data of accessed CBB galleries were tallied as 'zero' (not ented into the gallery) and one (ented into the gallery). The best models were compared against null model to attest possible random patterns in our predictor variables (Crawley 2007). To analyze the CBB predation by green lacewings we used a descriptive analysis, because on the first instar the predation results were the same. To survivorship of green lacewing in the presence of bored berries or not, was evaluated by using a survreg function from survival package v.3.1-8 (Therneau 2015). We also performed the contrast analysis using the lsmeans function from lsmeans package v.2.30-0 (Lenth 2016) to identify the levels in which the differences occurred. To analyze the CBB infestation and mortality due to presence and absence of a green lacewing, we conducted an analysis of variance (ANOVA), using generalized linear models (GLMs) with binomial errors distribution. The best-models were compared against null models to attest possible random patterns in our predictor variables. All analyses were performed using R 3.6.0 software (R Development Core Team, 2021).

3 Results

3.1 Access of Chrysopidae larvae to CBB gallery

We found that only the first instar of both green lacewing species entered in the galleries of CBB. The maturation stage of berries did not influence the preference of the green lacewing to enter the bored berries, either for *C. externa* (χ^2 = 1.074, df = 2, p = 0.58) (Fig. 3) or for *C. cubana* (χ^2 = 4.524, df = 2, p = 0.10) (Fig. 4).

The *C. externa* entered in 53.3% of the green bored berries, 60.0% of the ripe bored berries and 46.7% of the dry bored berries, averaging 53.3% of the entrance in the three maturation stages. During the evaluation we observed larvae *C. externa* removing and preying the of CBB eggs and larvae in the green bored berries. Larvae *C. cubana* entered 13.3% of the green bored berries, 10.0% of the ripe bored berries and 30.0% of the dry bored berries, averaging 17.7% of the entrance in the three maturation stages. We did not observe the removal of CBB from the berries by *C. cubana.*

3.2 CBB predation by *Chrysoperla externa* and by *Ceraeochrysa cubana*

First instar larvae of *C. externa* preyed on 100% of CBB eggs, larvae and pupae offered out of the berries, however, they did not prey on the adult CBB (Fig. 5) and the third instar larvae preyed on 76.7% of CBB adult. The same occurred with first larvae of *C. cubana* that preyed on 100% of CBB eggs, larvae and pupae and did not prey on the adult offered out of the berries (Fig. 5). Third instar larvae of *C. cubana* preyed only 40% of CBB adult.

3.3 *Chrysoperla externa* and *Ceraeochrysa cubana* survival on bored berries

The survival of *C. externa* and *C. cubana* followed the same pattern. In the presence of green bored berries without CBB adult at the entrance of the gallery, their larva survival was higher than in the presence of intact green berries (*C. externa*: $t = 14.592$, $df = 1$, $p < 0.05$ and *C. cubana:* $t = 2.578$, $df = 1$, $p < 0.05$) or no food (negative control) (*C. externa*: $t = 15.003$, $df = 1$, $p < 0.05$ and *C. cubana:* $t = 4.457$, $df = 1$, $p < 0.05$) (Table 1 and. 2). Survival of predators *C. externa* and *C. cubana* in intact green berries did not differ from the negative control (*C. externa*: $t = 0.355$, $df = 1$, $p > 0.05$ and *C. cubana:* $t = 1.655$, $df = 1$, $p > 0.05$). The positive control (eggs from *A. kuehniella*) provided greater survival compared to other treatments (Fig. 6 and 7) (Table 1 and. 2).

During the evaluations, we observed that 16 individuals of *C. externa* the molt to the second instar (53.3%) and three individuals for to the third instar (10%). Two larvae of *C. cubana* the molt to the second instar (6.67%).

3.4 CBB infestation in the presence of *Chrysoperla externa* and *Ceraeochrysa cubana*

The presence of third instar larva of *C. externa* decreased in 10% the number of green berries bored by CBB ($z = 2.276$, $df = 1$, $p < 0.001$) (Fig. 8). However, the infestation of CBB did not differ from the presence third instar larva of *C. cubana* ($z = 0.279$, $df = 1$, $p = 0.78$) (Fig. 9). The mortality of CBB adults was higher in the presence of *C. externa* than in the absence ($z = -3.102$, $df = 1$, $p < 0.001$) (Fig. 10), but was not influenced by the presence of *C. cubana* ($z = -1.511$, $df = 1$, $P = 0.131$) (Fig. 11)

4 Discussion

The first instar larvae of *C. externa* and *C. cubana* enter in the galleries of CBB in coffee berries in the different maturation stages (green, ripe and dry). However, when we compare the percentage of larvae that access the galleries, *C. cubana* had lower entrance ratio. We believe that the trash-carrying behavior may limit the ability of the *C. cubana* to access the galleries of bored berries, due to body size that increases with the presence of trash, impairing the access to the gallery which size is limited to 1.0 mm (Baker et al. 2002; Alba-Alejandre et al. 2018). Other factor that may have interfered in the entrance for both *C. cubana* and *C. externa* is the habit of the CBB adult to stay stopped at the gallery entrance which prevents access to any natural enemy (Pantoja 2018). In this experiment, we did not use X-ray to detect the CBB adult at the gallery entrance. However, the collection of bored berries was carried out at random and we opened the berries to confirm the presence of CBB immatures.

After verifying that green lacewings are able to enter the bored berries, we also found that the first instar larvae (*C. cubana* and *C. externa*) prey on CBB eggs, larvae and pupae, outside the fruit, but did not prey on adults. Only third instar larvae (*C. cubana* and *C. externa*) prey CBB adult outside the fruit. Green lacewings are generalist predators capable of feeding on various pests, mainly of soft-bodied (Costa et al. 2012; Oliveira et al. 2014; Tapajós et al. 2016; Nunes et al. 2017), which can explain the preference of green lacewing in feeding by immature stages of CBB. Possibly the size and fragility of the mouthparts of the early instars make in difficult perforation and predation of adults, since adults of CBB are hard, as they belong to the order Coleoptera having the sclerotized wings (Casari and Ide 2012). During the experiments, we observed that the third instar green lacewing larvae preyed on CBB adults feeding on the hemolymph, after piercing the more fragile parts of the CBB exoskeleton between the head and thorax. Thus, larvae green lacewing when prey the immature stages of CBB prevent the emergence of adults. Besides that, green lacewing when prey adults of CBB can limit the colonization of coffee berries.

The survival of green lacewings (*C. cubana* and *C. externa*) increased with the presence of bored berries without CBB adult at the entrance of the gallery indicating that they are able to enter and feed on CBB immature stages inside the berries. In addition, it seems that green lacewing did not feed on healthy berries because they survived equally without food, although phytophagy may occasionally happens (Batista et al. 2017). We also found that 63.33% of *C. externa* and 6.67% of *C. cubana* were able to molt in the presence of bored berries. The survival test confirms the limitation of *C. cubana* in entering the berries, but also shows that those larvae (possibly smaller) that are able to enter, feed on the CBB inside the berries. Different insect species are food source for green lacewing larvae (i.e. aphid, mealybug thrips, insect eggs) allowing them to complete development up to the pupal stage (Cardoso and Lazzari 2003; Costa et al. 2012; Tapajós et al. 2016; Bezerra et al. 2017). CBB can be another food source for *C. externa* as it has allowed the change of instar. We believe that *C. externa* did not reach the pupal stage due to food limitation, because we were unable to standardize the amount of food inside the berries and the food source was not replaced during the survival test. The survival test was carried out after berries being observed with the x-ray, to guarantee prey presence and by identify bored berries with CBB immature phases and without CBB adult in the gallery, but it was not possible to exactly standardized prey numbers. However, it gave a more realist condition, when we compared to other studies about predator survival (ants, beetles, and thrips) feeding on CBB out of the berries (Armbrecht and Gallego 2007; Larsen and Philpott 2010; Rezende 2014; Follett et al. 2016; Morris and Perfecto 2016).

Third instar *C. externa* larvae prevented the CBB females from borer the berries and they were also able to prey on the CBB adults, but this not occurred for *C. cubana*. Studies show that trash-carrying larvae of Chrysopidae have this beheviour for protection (Canard and Duelli 1984; Tauber et al. 2000, 2014), while naked larvae need to be more agil and produce repellent secretions to defend themselves (Canard and Duelli 1984; Haruyama et al. 2012; Souza and Bezerra 2019). We believe that *C. externa* was more efficient in preventing the CBB from piercing the berries because of its behavior. During the experiment we observed that *C. externa* was more agile to look for prey and apparently more voracious. The same happen with some ants play the role of preventing CBB from entering the berries during its dispersion (Philpott et al. 2012; Gonthier et al. 2013).

By identifying the potential and differential predation of two lacewing species on CBB, we added them to the list of natural enemies of this important pest. Understanding interactions among prey and natural enemies would help in design more effective strategies to manage CBB populations. One strategy to keep green lacewings in coffee crops is the introduction of strategic plants to provide alternative resources to adults who feed on pollen and sugary foods of plant origin (Venzon and Carvalho 1992; Venzon et. al. 2006; Tauber et al. 2009). Besides that, the use of green lacewings as a curative measure would be possible when necessary, as the species is commercially available in Brazil.

In summary, we found that *C. externa* and *C. cubana* are capable of prey the CBB inside the berries. Third instar larvae of *C. externa* prey on CBB females and limited their colonization of coffee berries that may contribute to reduce losses caused by CBB.

5 Acknowledgments

We thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café (CBP & D-Café) for research grants and fellowships to the authors. We thank AMIPA (Associação Mineira dos Produtores de Algodão) for providing *C. externa* eggs for continuing our laboratory rearing for experiments.

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Tables

Table 1 Comparison of the survival of *Chrysoperla externa* in the presence of different food resources. The statistical values represent the contrast analysis (t test) between resources. Significant values (p ≤0.05) are in bold.

Paired comparison	**Estimate [±EP]**	**t.ratio**
GBB *versus* HGB	0.877± 0.0601	**14.592**
GBB *versus* Negative control	0.893± 0.0595	**15.003**
GBB *versus* Positive control	-0.560± 0.0630	**8.886**
HGB *versus* Negative control	0.016 ± 0.0454	0.355
HGB *versus* Positive control	-1.437± 0.0500	**28.734**
Negative control *versus* Positive control	-1.453± 0.0493	**29.472**

Paired comparison between: healthy green berries (HGB); green bored berries (GBB); *A. kuehniella* eggs (Positive control); and without food (Negative control).

Table 2 Comparison of the survival of *Ceraeochrysa cubana* in the presence of different food resources. The statistical values represent the contrast analysis (t test) between resources. Significant values (p ≤0.05) are in bold.

Paired comparison	**Estimate [±EP]**	**t.ratio**
GBB *versus* HGB	0.205 ± 0.0796	**2.578**
GBB *versus* Negative control	0.335 ± 0.0753	**4.457**
GBB *versus* Positive control	-1.282 ± 0.0722	**17.764**
HGB *versus* Negative control	0.130 ±0.0787	1.655
HGB *versus* Positive control	-1.487 ± 0.0757	**-19.653**
Negative control *versus* Positive control	-1.618 ± 0.0712	**-22.710**

Paired comparison between: healthy green berries (HGB); green bored berries (GBB); *A. kuehniella* eggs (Positive control); and without food (Negative control).

Figures

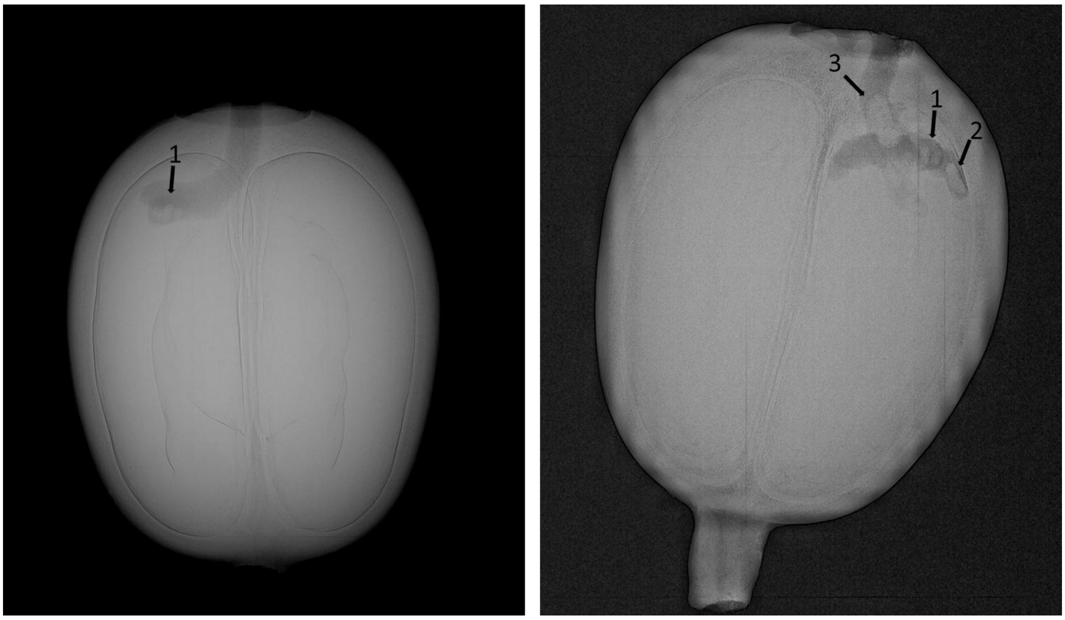

Figure 1 X-ray image (Faxitron® LX-60 cabinet) of the interior of the green bored berries, showing the presence of CBB eggs (1), larva (2) and the adult at the entrance of the gallery (3).

Figure 2 Arenas using a transparent Gerbox (11 cm long x 11 cm wide x 3.5 cm high) covered with PVC film, containing a thin wooden rod (11 cm long x 3 mm in diameter) and five green coffee berries without CBB infestation fixed with hot glue on it, mimicking a coffee rosette.

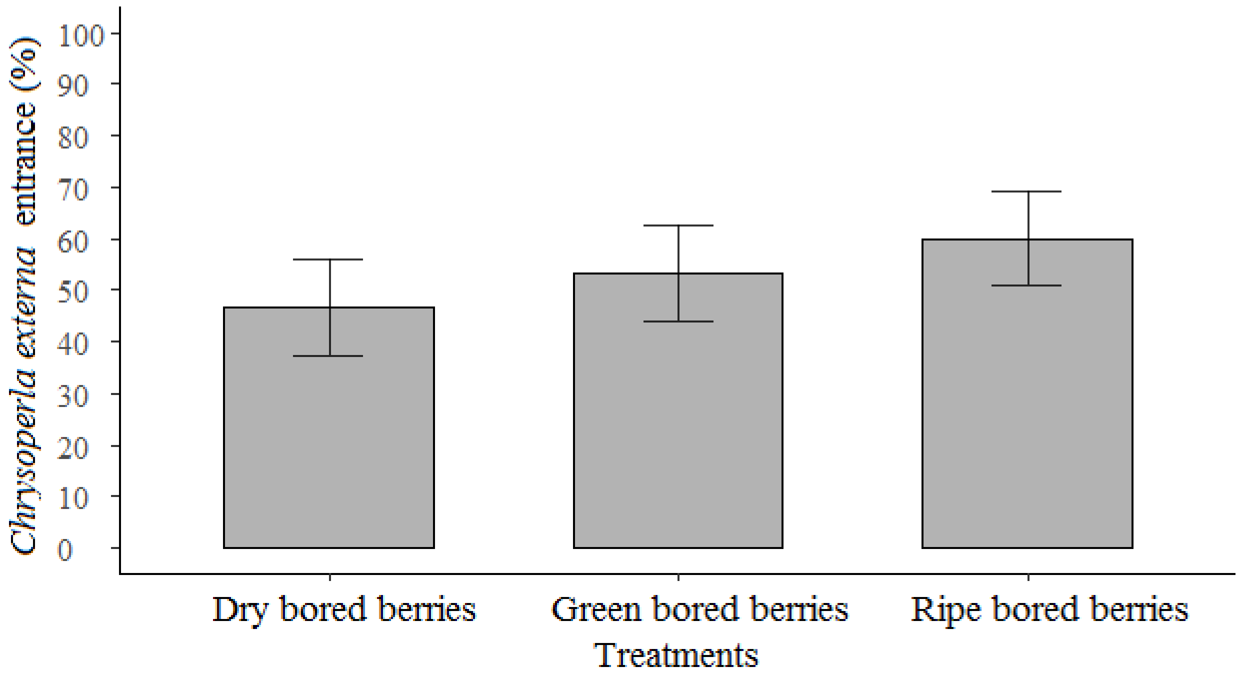

Figure 3 Proportion of the response of first instar *Chrysoperla externa* larvae to enter in bored berries at different maturation stages: green, ripe and dry ($\chi 2 = 1.074$, df = 2, p = 0.58).

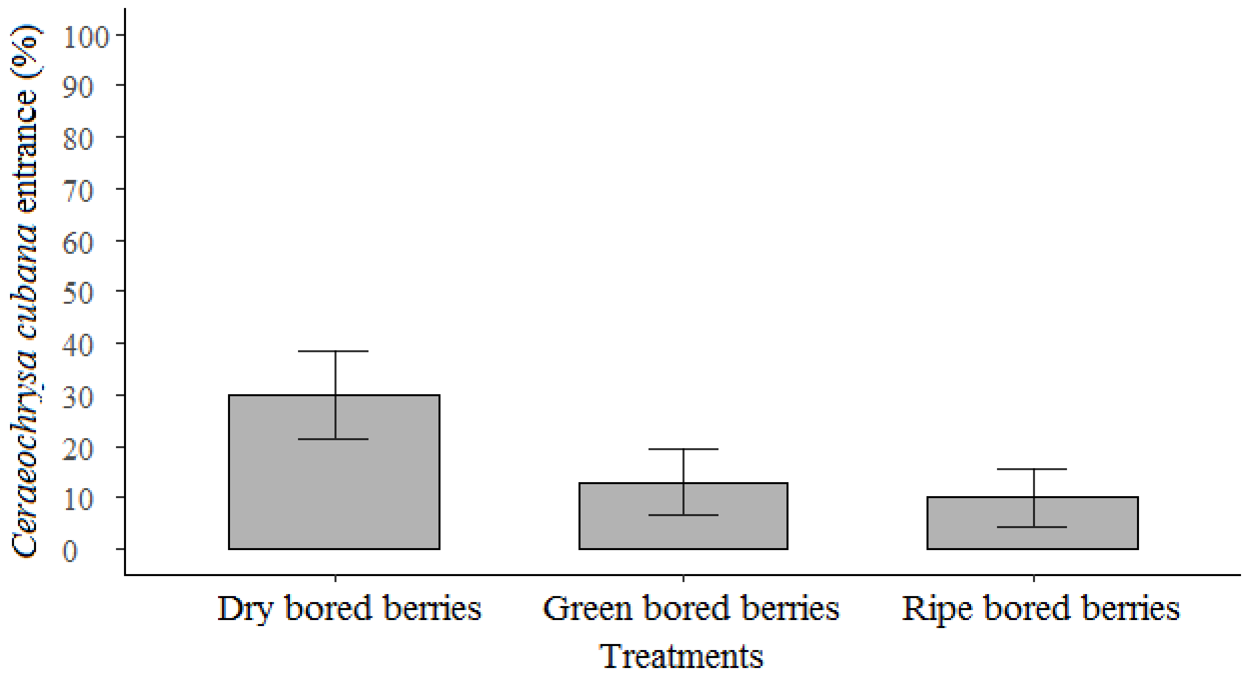

Figure 4 Proportion of the response of first instar *Ceraeochrysa cubana* larvae to enter in bored berries at different maturation stages: green, ripe and dry ($\chi 2 = 4.524$, df = 2, p = 0.10).

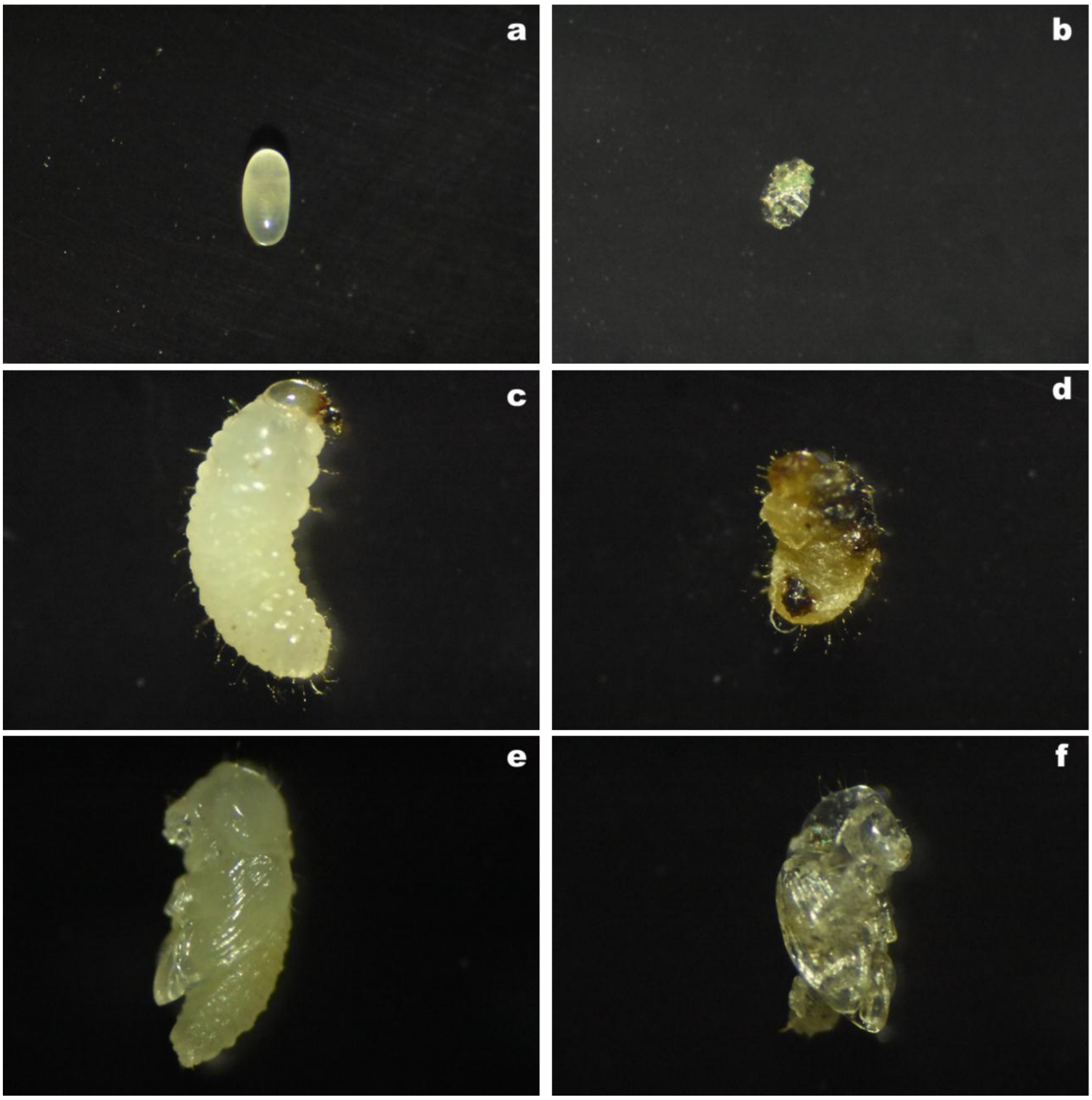

Figure 5 CBB egg (a), larva (c), and pupa (e) removed of bored berries. Preyed egg (b), larva (d), and pupa (f) of CBB by green lacewing.

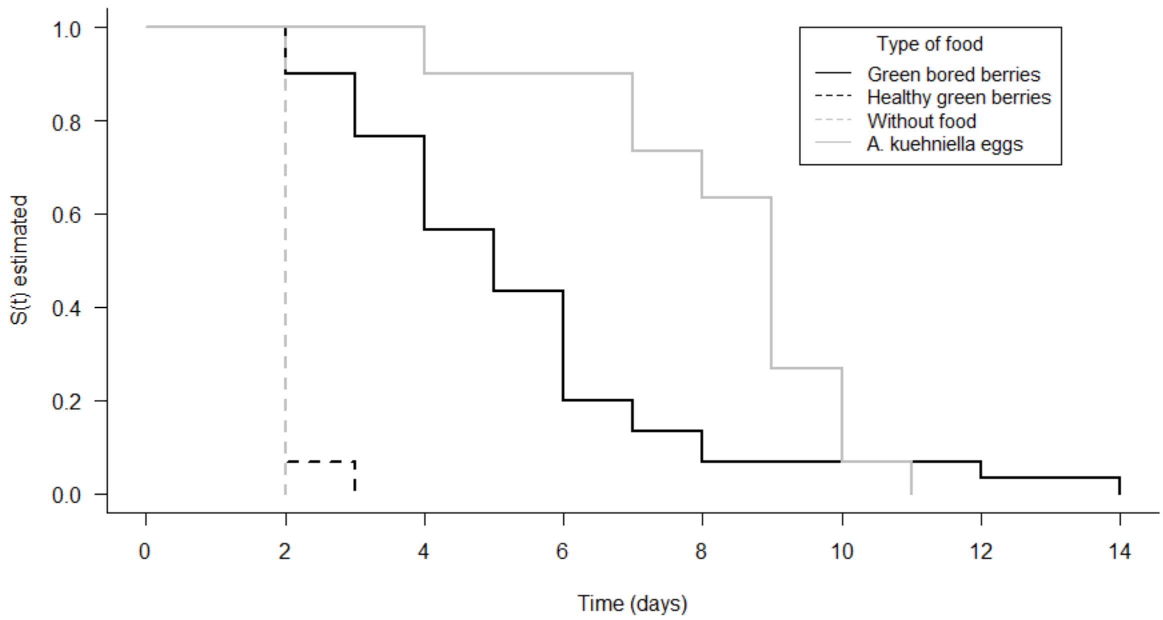

Figure 6 Survivorship of *Chrysoperla externa* along the time in the presence of either healthy green berries, green bored berries, *A. kuehniella* eggs (Positive control), or without food (Negative control).

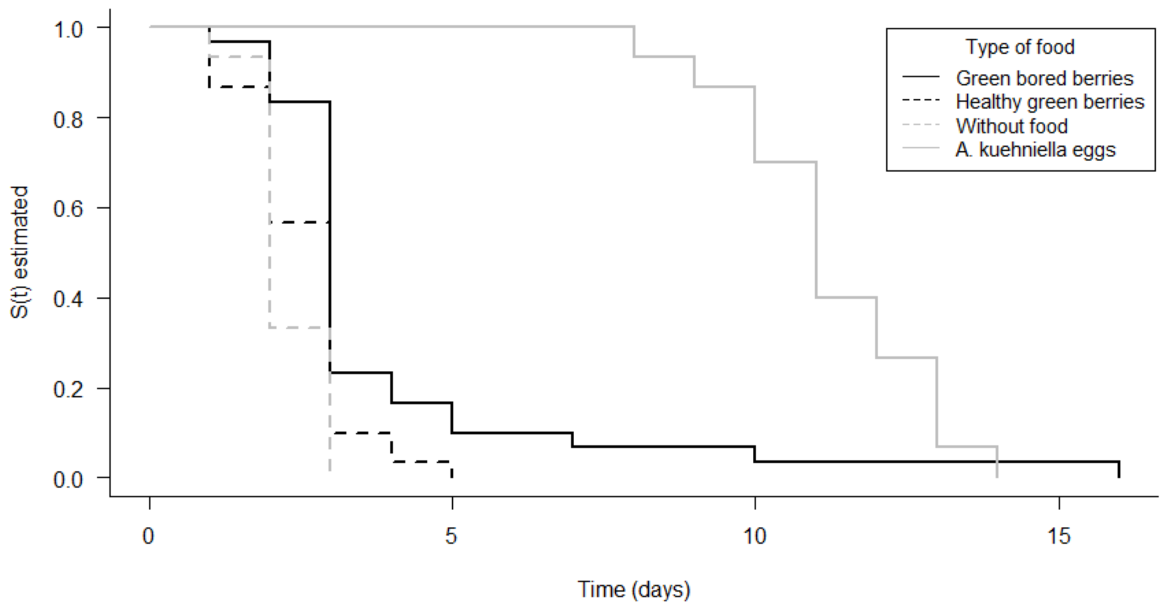

Figure 7 Survivorship of *Ceraeochrysa cubana* along the time in the presence of either healthy green berries, green bored berries, *A. kuehniella* eggs (Positive control), or without food (Negative control).

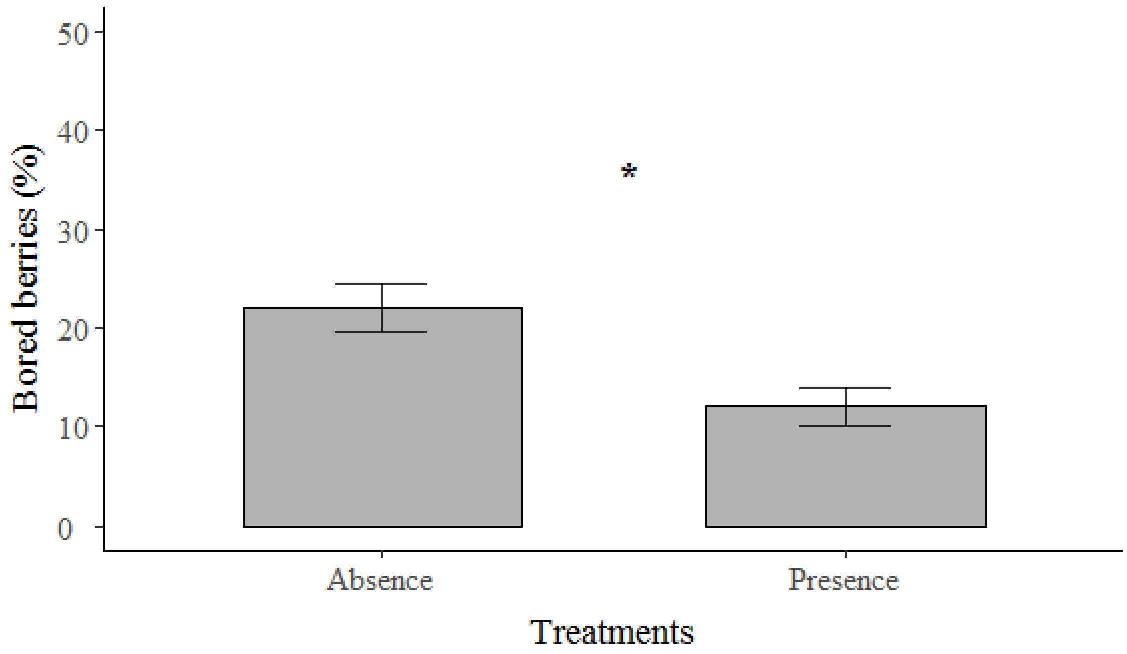

Figure 8 Average (± se) of percentage of bored berries by CBB in the presence and absence of third instar *Chrysoperla externa* larvae (z = 2.276, df = 1, p <0.001). The asterisk represents the significant difference between the bars.

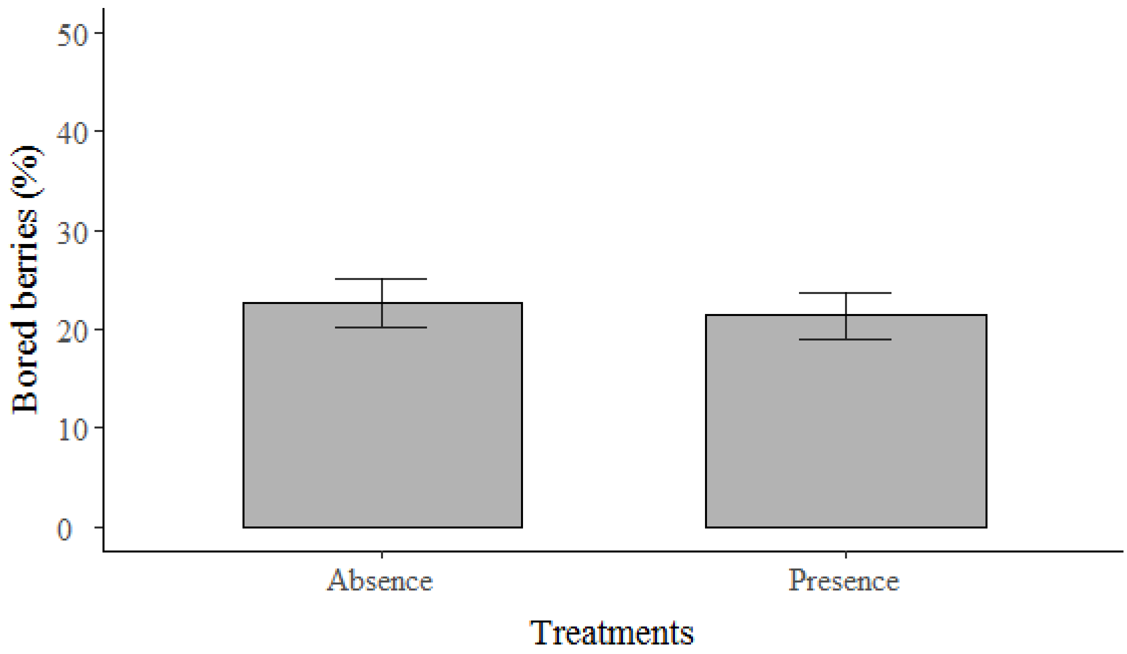

Figure 9 Average (± se) of percentage of bored berries by CBB in the presence and absence of third instar *Ceraeochrysa cubana* larvae (z = 0.279, df = 1, p = 0.78).

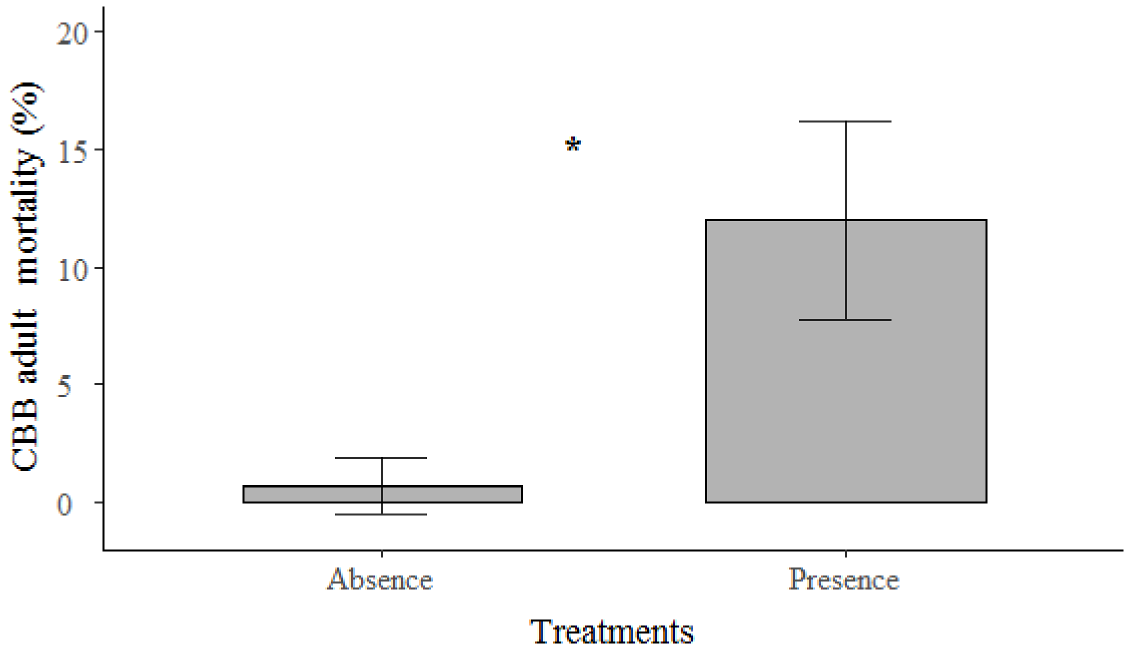

Figure 10 Average (± se) of percentage CBB adult mortality in the presence and absence of third instar *Chrysoperla externa* larvae (z = -3.102, df = 1, p <0.001). The asterisk represents the significant difference between the bars.

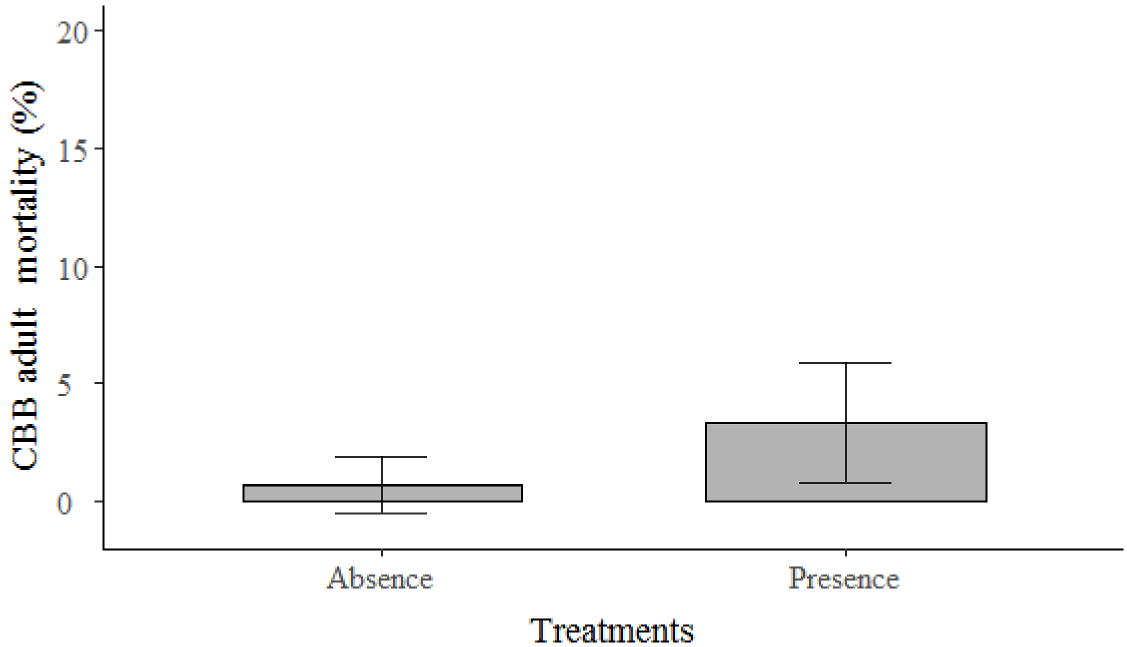

Figure 11 Average (± se) of percentage CBB adult mortality in the presence and absence of third instar *Ceraeochrysa cubana* larvae (z = -1.511, df = 1, P = 0.131).

Chapter III[1]

Diversified coffee system as a strategy to increase abundance and richness of coffee berry borer predatory ants.

Abstract

Conventional coffee crop management includes simplified monoculture agroecosystems, resulting in natural enemy loss. A tool to diminish this problem is to increase biodiversity in the coffee crop by association with plants that attract and provide resources to natural enemies. Here, we designed a diversified coffee system and investigated if this strategic diversification enhanced the richness and abundance of predatory ants in the crop, resulting lower populations of the coffee berry borer (CBB), *Hypothenemus hampei*. The diversified system had *Inga edulis*, *Senna macranthera*, *Varronia currasavica* and non-crop plants associated to coffee plants. We collected ants and evaluated the predation rate by using live-bait traps (*Tenebrio molitor*). We also evaluated the infestation of CBB and the coffee production. The diversified system had greater richness and abundance of predatory ants, higher predation rate and lower percentage of bored coffee fruits compared to conventional coffee system. Besides, the coffee yield did not differ between the systems. Our results suggest that strategic diversification increases predatory ants in coffee crop, resulting in the natural control of CBB.

Keywords: *Inga edulis, Senna macranthera*; *Varronia currasavica*; Conservation biological control; Predatory ants; Coffee berry borer

[1] Chapter formatted in the norms of the Journal Agriculture, Ecosystems and Environment.

1 Introduction

Predatory ants are important biological control agents in agroecosystems (Philpott and Armbrecht, 2006; De la Mora et al., 2015; Morris et al., 2018). Studies report that ants have potential to reduce levels of pest infestation in different crops (De la Mora et al., 2015; Morris et al., 2018). In coffee crops, the ants are reported as important natural enemies of coffee berry borer (CBB), *Hypothenemus hampei*, due to their attack on CBB eggs, larvae, pupae and adults inside the berries, decreasing CBB survival (Bustillo et al., 2002). In addition, large ants can serve as patrollers, attacking and preventing CBB from entering the berries and reducing damage, without consuming them (Way and Khoo, 1992; Philpott et al., 2008). The CCB is the major coffee pest worldwide, because attack coffee fruits, causing yield and quality coffee losses. The economic losses by CCB attack may be estimated at US$215-358 million a year in Brazil (Oliveira et al., 2013).

The landscape simplification in coffee monocultures can lead to a decrease in richness and abundance of ants that promote CCB biological control (De la Mora et al., 2013; Perfecto and Vandermeer, 2002). On the other hand, the coffee association with other plant species can favor the maintenance of ants in coffee crops, through the provision of resources such as alternative food and shelter (Amaral et al., 2013, Rezende et al., 2014). But this association must be carried out strategically in order to improve the biological control service (Meyer et al., 2009). For instance, plants that have extrafloral nectaries and/or present constant blooms are potentially candidates in diversification process, because they can provide food, such as nectar and pollen, for several natural enemies such ants (Koptur, 1994; Souza et al., 2010; Rezende et al., 2014). Besides that, the plants associated should not host the key pests of the crop. In the case of the CBB, this is not a problem, due to as there is no evidence that alternate hosts are used as a food source or place of reproduction (Vega et al., 2015; Vega et al., 2020). In addition, it is essential that the associated plants do not compete for water and nutrients with the coffee plants and that they are easy to manage (Souza et al., 2010). Thus, in this study we selected three plant species to associate with coffee crops: (1) *Inga edulis* (Fabaceae), an extrafloral nectary tree with high potential to fix nitrogen in soil (Souza et al., 2010; Tully et al., 2012; Rezende et al., 2021); (2) *Senna macranthera* (Fabaceae), a commonly tree species used in *C. arabica* agroforestry systems (Souza et al., 2010) and that it also has extrafloral nectaries (Marazzi et al., 2013); and (3) *Varronia currasavica* (Cordiaceae), a perennial native shrub with medicinal properties that blooms all year round, providing a constant source of pollen and nectar (Brandão et al., 2015; Martins, 2017; Hoeltgebaum et al., 2018). The diversification was also stimulated through the maintenance of non-crop plants, due to provision of alternative food and refuge for natural enemies (Amaral et al., 2013; Fonseca et al., 2017; Togni et al., 2019; Venzon et al., 2019).

Thus, our aim was to investigate whether strategic diversification in coffee crops increases biological control of CBB by predatory ants and coffee yield, through the association the *I. edulis*, *V. curassavica*, *S. macranthera* and non-crop plants. Our hypotheses are that the strategic coffee crop diversification (i) increases the richness and abundance of predatory ants, (ii) increases predation activities, (iii) contributes to reduction of the CBB infestation rate, and (iv) increases coffee production.

2 Materials and Methods

2.1 Study area and Sampling design

Experiments were conducted at Experimental Research Station of Agriculture and Livestock Research Enterprise of Minas Gerais (Epamig) in Patrocínio - MG, Brazil (18 ° 59’48’’S, 46 ° 59’00’’W, 934 meters elevation). Located in the Cerrado biome, the region presents the Aw climate, according to the Köppen classification, with two distinct seasons, dry winter and rainy summer, and an average annual precipitation of 1,620.1 mm (Silva and Malvino, 2005). The terrain is flat to roll smoothly, with an average slope of 3% and the soil is classified as Red-Yellow Latosol (Latosol), according to the Brazilian Soil Classification System (Kamimura et al., 2020).

The treatments were represented by the coffee systems: (1) Diversified coffee system and (2) Conventional coffee system. The treatments were arranged in three blocks. Two blocks are of the “Catuaí Vermelho IAC 99” variety and one of the “Acaiá IAC 474 - 19” variety, with 4m between lines and 0.5 m between plants. The blocks of the Catuaí variety were installed in 1993 and 1994, and the block of the Acaiá variety were installed in 1987 with truking (with the plant cut at about 30 - 40 cm from the ground) in 1998. The cultivars have the same susceptibility to the most pests and diseases of coffee plants (Fazuoli et al., 2007). Each plot has an area of 1,080 m^2 and were separated from each other by 200 m. The minimum distance between blocks was 500 m (Fig. 1).

In each diversified coffee plot, coffee was associated with four plants of *I. edulis*, 12 plants of *V. curassavica*, and two plants of *S. macranthera*. The *I. edulis* and *S. macranthera* were obtained at the Espaço Botânico greenhouse at Uberlândia-MG, Brazil. These seedlings were approximately 80 cm high when them were transplanted. The *V. curassavica* seedlings were produced from seeds harvested at Experimental Research Station of Epamig, in Oratórios-MG, Brazil. Seedlings of *V. curassavica* were transplanted with approximately 30 cm high. The transplant of the three species was carried out in December 2018. The plants were inserted in two lines, one at each borders of the plots with a spacing of 5 m between plant (Fig. 2).

In the diversified coffee system plots, non-crop plants were kept between the lines at a height of 50 cm. When necessary, handling was done with mechanical suppressed. Pesticides were not applied. In the conventional coffee system, coffee was grown in monoculture with chemical and mechanical management of non-crop plants and application of pesticides for the management of pests and diseases. In both systems, mineral fertilization was maintained under standard coffee management of the Cerrado (Appendix A, B and C). The coffee harvesting in both systems was carried out in mechanized manner in June 2019, 2020 and 2021. However, for production data collection, the plants were harvested manually. The repase, a technique where the maximum amount of coffee berry is removed from the ground (Johnson et al., 2019; Constantino et al., 2021), was carried out in October 2019 and September 2020. In our areas, the repase in a mechanized manner. First, all organic material is transferred to the middle of the space between lines of coffee by the Miac ASM 2H machine. Then, the coffee swept from ground ("varreção") by the Miac Master Café 2 machine, where the coffee berries are separated from the rest of the organic material.

2.2 Richness and abundance of predatory ants

In order to compare the richness and abundance of predatory ants in the diversified and the conventional systems. Samples in the diversified systems were collected in different distances (4, 8, 12 and 16 m) from the introduced plants, in three points in each row having the introduced plants as origin. The first point in front of *I. edulis*, second point in front of *V. currasavica* and the third point in front of *S. macranthera* by eigth rows, totally 24 samples per plot. In the conventional system, collection was also carried out on 24 plants, following the pattern of three plants per row in eight rows. Each trap consisted of one transparent pot (250ml) with eight 0.5 mm holes made around the wall of each pot (close to the lid)(Fig. 3). Inside the trap, we placed a small vial (10 ml) with three live *Tenebrio molitor* larvae (Coleoptera: Tenebrionidae) of approximately 4.0 cm (Fig. 3). The larvae were used as prey to attract predatory ants (Pacheco et al., 2017). We filled the space between the vial and the pot inner wall with water and neutral detergent (9:1). We placed each trap in the soil were buried until the holes were close to the ground, approximately 5.0 cm, under the coffee canopy, with at least

5 meters of distance between traps (Fig. 3). The traps remained in the field for two days (Ribas et al., 2003; Silva et al., 2011). After that, we collected the insects and kept them in 70% alcohol until identification and count the number of predatory ants to determine their abundance.

2.3 Predation in different areas using live-bait trap

To estimate ant predation rates, we use the live-bait trap methodology developed by Pacheco et al. (2017). The traps were composed of a transparent pot (250ml) closed with a lid and containing one live *T. molitor* larva of approximately 0.5 cm. The trap had eight 0.5mm holes made around the wall of each pot. This method prevents *T. molitor* from climbing the pot walls, while allowing ants to enter and exit more easily, because the larvae cannot climb on smooth surfaces (Casari and Ide 2012). The traps were placed in the soil, under the canopy of 24 coffee plants per plot. They were buried until the holes were close to the ground, approximately 5.0 cm. The traps remain in the field for 48 hours. Subsequently, we removed the traps and visually checked larvae predation. We consider predation as partially consumed larvae or absent larvae. All individuals found in the pots were collected and stored in 70% alcohol for identification.

2.4 Coffee berry borer infestation rate

We evaluated the infestation rate of CBB in all plots of both systems. For this, we collected coffee berries following the methodology adapted of Souza and Reis (1997). We collected 20 coffee berries per plant, from the central portion of the plants, 10 berries in the east and 10 in the west position. The sampling was carried out in 48 plants randomly chosen per plot, in six plants per line, in eight lines. Sampling was carried out in March, May and June 2019, January and June 2020, and February, March and April 2021. Then, we counted the number of bored berries per plant.

2.5 Coffee yield

We estimated the coffee yield by collecting all berries from 24 coffee plants on each plot. The harvest was carried out in Jun 2019, 2020 and 2021. However, we did not use de yield of 2019, because conventional agronomic practices were maintained in the experimental area until December 2018, when we installed the experiment. Therefore, the coffee harvest in 2019 was influenced by 2018 management, when occurred the bloom and coffee fruit formation. Handling coffee harvesting in both systems was carried in the same day. In total, 72 coffee plants were sampled to assess coffee yield in each year per system. To estimate the production, we measure the volume of coffee fruits of each plant (L/plant) with the help of a graduated bucket. In addition, we collected and stored 100 berries from each plant (g/100 fruits per coffee plant). In addition, we collected and stored 100 berries from each plant (g/100 fruits per coffee plant). On the same day of collection we weighed the 100 fruits in laboratory with the help of an analytical balance to estimate the weight of the fruits.

2.6 Statistical analysis

We tested the variation in richness and abundance of predatory ants using GLMM with a negative binomial error distribution. In the model, the systems (diversified and conventional) were defined as a fixed effect and a block as a random effect. The GLMM was compared against null models to attest possible random patterns in the predictor variables. To estimate the efficiency of collections we use the collector curve using an iNEXT function from iNEXT package v.2.0.20 (Hsieh et al., 2016).

To assess the percentage of live-bait predation in the different systems, we used GLMM with binomial error distribution. In the model, the systems (diversified and conventional) were defined as a fixed effect and a block as a random effect. The GLMM was compared against null models to attest possible random patterns in the predictor variables.

To evaluate the percentage of bored berries in the two systems (diversified and conventional), we used the Mixed Generalized Linear Model (GLMM) with binomial error distribution. In the model, the systems and years (2019, 2020 and 2021) were defined as fixed effects and block as a random effect. The GLMM was compared against null models to attest possible random patterns in the predictor variables (Crawley, 2007). The GLMMs were performed with glmmTMB package, v.1.0.2.1 (Brooks et al., 2017).

To evaluate the coffee yield in different years, we used GLMM with negative binomial error distribution. In the model, the systems (diversified and conventional) were defined as fixed effects and block as a random effect. The GLMM was compared against null models to attest possible random patterns in the predictor variables. All analyzes were performed in R version 3.6.3 (R Core Team 2020).

3 Results

3.1 Richness and abundance of predatory ants

The collector curve showed that we collected 86.7% of the species in the diversified system and 84.6% in the conventional system, showing that the sampling effort was adequate to identify the ant community (Fig. 4). We recorded 26 species of predatory ants in the diversified system (3340 individuals), belonging to subfamilies Myrmicinae, Dolichoderinae, Ectatomminae, Ponerinae, Formicinae and Pseudomyrmecina. In the conventional system, we recorded 22 species (2284 individuals) belonging to subfamilies Myrmicinae, Dolichoderinae, Ectatomminae, Ponerinae, Formicinae and Pseudomyrmecina (Table. 1). The richness of predatory ants was higher in the diversified coffee system than conventional coffee system ($\chi 2$

= 25.5032, df = 1, p <0.001) (Fig.5). The species *Pheidole* sp.12, *Pheidole* sp.13, *Pheidole* sp.14, *Solenopsis* sp.2, *Pachycondyla striata, Strumigenys louisianae* and *Anochetus* sp.1 were found only in the diversified system (Table 1). The species *Pheidole* sp.4, *Octostruma* sp.1 and *Carebara* sp.1. were found only in the conventional system (Table. 1). The abundance of predatory ants was also higher in the diversified coffee system than conventional system ($\chi2$ = 18.134, df = 1, p <0.001) (Fig. 6). The most abundant genera were *Pheidole* and *Solenopsis* on both systems (Table. 1).

3.2 Predation in different areas using live-bait trap

We found higher predation of live-baits in the diversified system than in the conventional ($\chi2$ = 8.668, df = 1, p <0.05) (Fig. 7). The average predation rate in the diversified system was 73.61% whereas the conventional system it was 62.85%. The most common arthropods found inside traps were predatory ant (80%) in both systems (Table. 2).

3.3 Coffee berry borer infestation rate

We found highest rate of CBB infestation in the diversified system in 2019 (t = -12.704, p <0.001) (Fig. 8) compared to conventional system. However, the proportion of bored berries was lower in the diversified system than in the conventional system, in 2020 (t = 5.330, p <0.001) and 2021(t = 7.621, p <0.001) (Fig. 8).

3.4 Coffee yield

We did not find difference in weight of fruits (N = 100) between diversified and conventional systems in 2020 and 2021 ($\chi2$ = 0.856, df = 1, p =0.354) (Fig. 9). We also did not find difference in volume of coffee fruits of each plant between diversified and conventional systems in both years ($\chi2$ = 0.962, df = 1, p =0.326) (Fig. 10).

4 Discussion

Our results show a greater richness and abundance of predatory ant species in the diversified system. The most abundant genera were *Pheidole* and *Solenopsis* in the diversified and conventional systems. Both are described as CBB predators (Bustillo et al., 2002; Armbrecht and Gallego, 2007; Aristizábal and Metzger, 2019) and they are reported to feed on extrafloral nectar of *Inga* sp. (Rezende et al., 2014). In addition, ants are commonly found associated with *V. curassavica* due to the constant presence of flowers (Brandão et al., 2015; Martins, 2017; Hoeltgebaum et al., 2018). Studies, in laboratory, show that *Solenopsis* ants prey on immature CBB inside the berries (Morris and Perfecto, 2016) and remove CBB adults from the berries (Armbrecht and Gallego, 2007; Larsen and Philpott, 2010). Another study shows that different species of generalist ants, including species of *Pheidole*, defend coffee plants from CBB infestation (Gonthier et al., 2013; Larsen and Philpott, 2010). In this work, we observed the presence of ants feeding on extrafloral nectar de *I. edulis* and *S. macranthera* and on floral nectar of *V. curassavica* (data not shown). We believe that the presence of associated plants influenced the attractiveness and maintenance of these ants, due to the constant supply of carbohydrate food source. Besides that, non-crops plants in the coffee space between lines may have contributed to the maintenance of CBB natural enemies including ants, due to the provision of resources such as food and shelter (Seguni et al., 2011).

We found the CBB infestation decreases in the diversified system compared to the conventional over time. Everything indicates that the presence of predatory ants contributed to the reduction of CBB infestation. A recent study showed that the presence of ants (e.g.

Cephalotes sp., *Linepithema* sp., *Pheidole* spp., *Solenopsis* sp., *Crematogaster* sp., *Camponotus* sp., *Neoponera* sp., *Brachymyrmex* spp.) decreased CBB infestation in coffee crops near forest fragments (Aristizábal and Metzger, 2019). The decrease in CBB infestation began after the 13th month of transplanting of associated plant species. In the process of transition from conventional to diversified systems, environmental gains such as biological control may take time to occur, since they depend on the ecological processes involved in the recovery and conservation of these areas (Gliessman, 2005; Siqueira et al., 2010). Possibly, the difference between the systems in the second and third year was due to the increase of the population of predatory ants and other natural enemies of CBB, as green lacewings and parasitoids. Martínez-Salinas et al. (2016) showed that different levels of unplanned diversification (forests, sugar cane, pasture) in coffee crops decrease CBB infestation (Martínez-Salinas et al., 2016). Rezende et al. (2021) showed that plants of *Inga subnuda* consorted to coffee decrease the number of bored berries due to the increase of natural enemies. Therefore, the diversification of the coffee crop contributes to the management of CBB.

The coffee fruit weight and the volume of liters per plant did not differ between the diversified and conventional systems in 2020 and 2021. The conventional system presented a higher production cost (Appendix 4) due to the application of insecticides, acaricides, fungicides and herbicides, which are not used in the diversified system. Besides that, the intensive use of these pesticides brings harm such as loss of biodiversity, environmental pollution, pest resistance, emergence of secondary pests, possible intoxication of coffee farms workers (Krishna et al., 2003;Yáñez and France, 2010; Janssen and Rijn, 2021). Therefore, diversified coffee systems contribute to the reduction of pesticide applications without negatively affecting coffee production. Finally, diversified coffee systems are economically profitable, and secure to the environmental and human health, which are financially immeasurable.

5 Acknowledgments

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café (CBP & D-Café) for research grants and fellowships to the authors. The staff of EPAMIG of Patrocínio, Jaime, Diego, Greice, Nenzin, Juliana, Marcela, coffee pickers is thanked, for all its support in carrying out the field experiments.

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Tables

Table 1 Identity and numbers of predatory ants collected in traps in conventional and diversified coffee plots in Patrocínio-MG.

Subfamilies	**Ant**	**Conventional**	**Diversified**	**Total**
Myrmicinae	*Pheidole gertrudae*	8	16	24
	Pheidole sp.1	52	50	102
	Pheidole sp.3	3	78	81
	Pheidole sp.4	2	0	2
	Pheidole sp.5	57	54	111
	Pheidole sp.6	259	237	496
	Pheidole sp.8	8	16	24
	Pheidole sp.9	55	57	112
	Pheidole sp.10	94	220	314
	Pheidole sp.11	563	1020	1583
	Pheidole sp.12	0	1	1
	Pheidole sp.13	0	48	48
	Pheidole sp.14	0	1	1
	Solenopsis saevissima	593	655	1248
	Solenopsis sp.1	5	10	15
	Solenopsis sp.2	0	43	43
	Octostruma sp.1	1	0	1
	Strumigenys louisianae	0	1	1
	Carebara sp.1	1	0	1
Formicinae	*Brachymyrmex* sp.1	24	9	33
	Brachymyrmex sp.2	4	8	12
	Camponotus sp.1	4	23	27
Ponerinae	*Pachycondyla striata*	0	1	1
	Odontomachus chelifer	8	5	13
	Anochetus sp.1	0	1	1
Dolichoderinae	*Linepithema* sp.1	132	422	554
	Dorymyrmex sp.1	301	286	587
Ectatomminae	*Gnamptogenys striatula*	109	75	184
Pseudomyrmecinae	*Pseudomyrmex* sp.1	1	3	4
Total		2284	3340	5624

Table 2 Groups and number of arthropods collected in traps in a conventional and diversified coffee system in Patrocínio-MG.

Groups	**Conventional system**	**Diversified system**
Predatory ants	153	200
Coleoptera	9	18
Araneae	21	19
Blattaria	1	-
Gryllidae	2	-
Bedbugs	-	2
Polydesmida	4	7
Cicadellidae	-	1
Chrysopidae	-	1
Total	190	248

Figures

Figure 1 Experimental area located at EPAMIG in Patrocínio-MG. The experiment had three blocks with two treatments, a diversified coffee system (continuous square) and a conventional coffee system (dashed square). Each plot measures 1080 m².

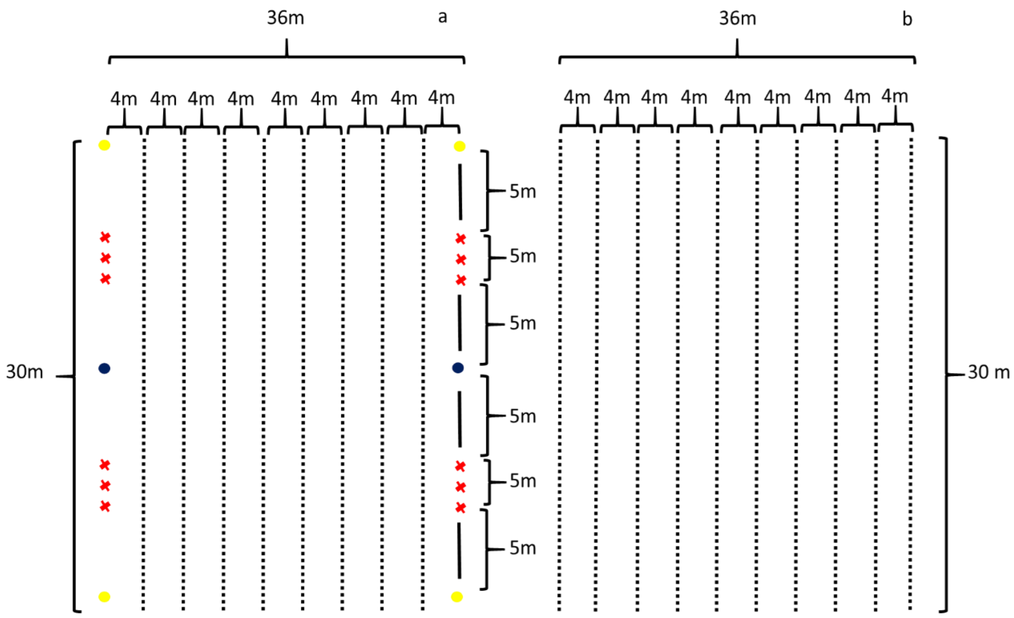

Figure 2 Diversified coffee system plot: yellow dots represent the *Inga edulis* plants, the blue ones the *Senna macranthera* plants and the red "x" the *Varronia curassavica* plants. Black dots represent coffee plants (a). Conventional coffee system plot: black dots represent coffee plants (b). Each plot has an area of 1,080 m².

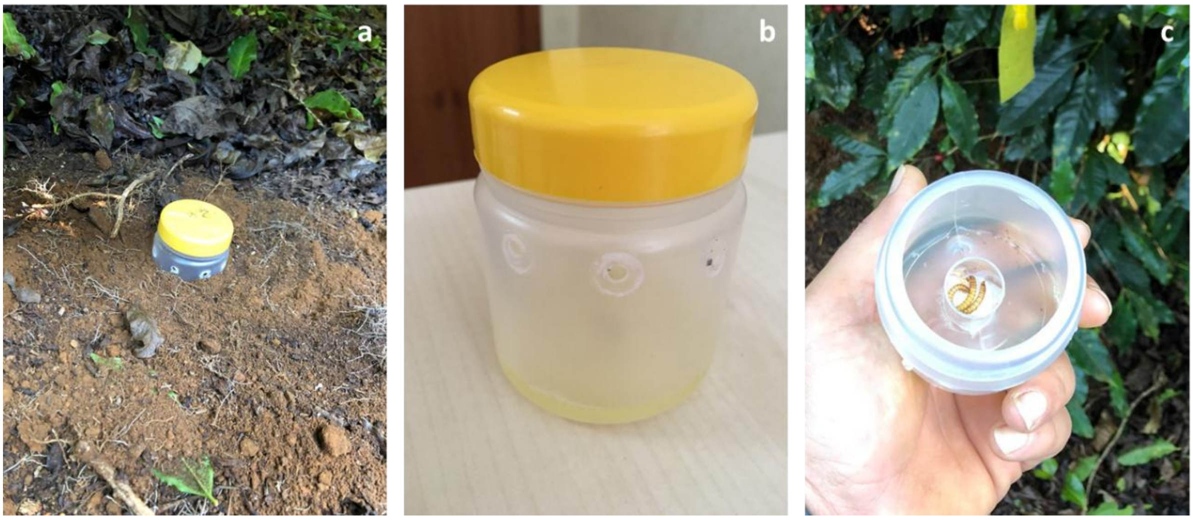

Figure 3 Live bait trap for collecting ants in different coffee systems.

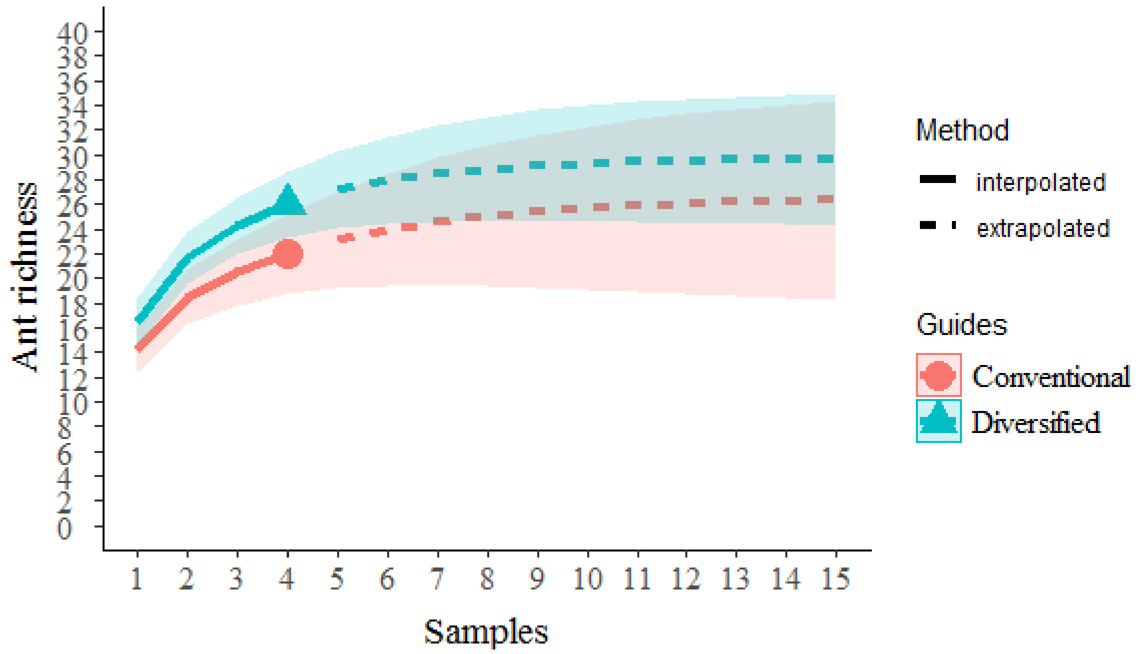

Figure 4 Collector curves showing the accumulated richness of predatory ants in a conventional and diversified coffee system.

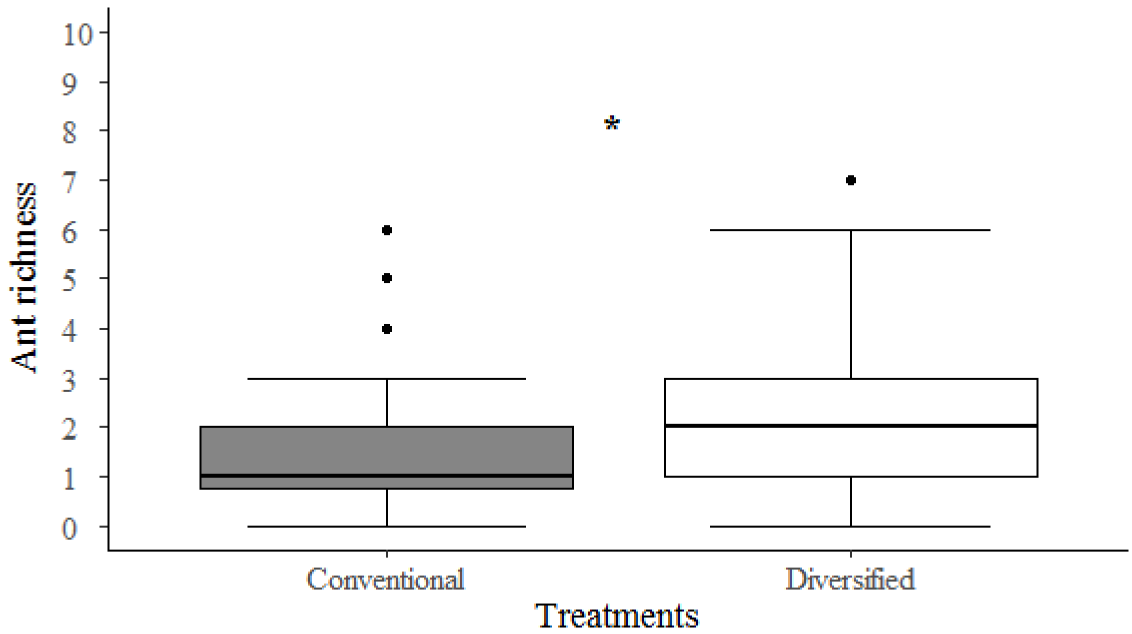

Figure 5 Box plot of the richness of predatory ants in a conventional (gray box) and diversified (white box) coffee system ($\chi 2$ = 25.5032, df = 1, p <0.001). The asterisk represents the significant difference between treatments.

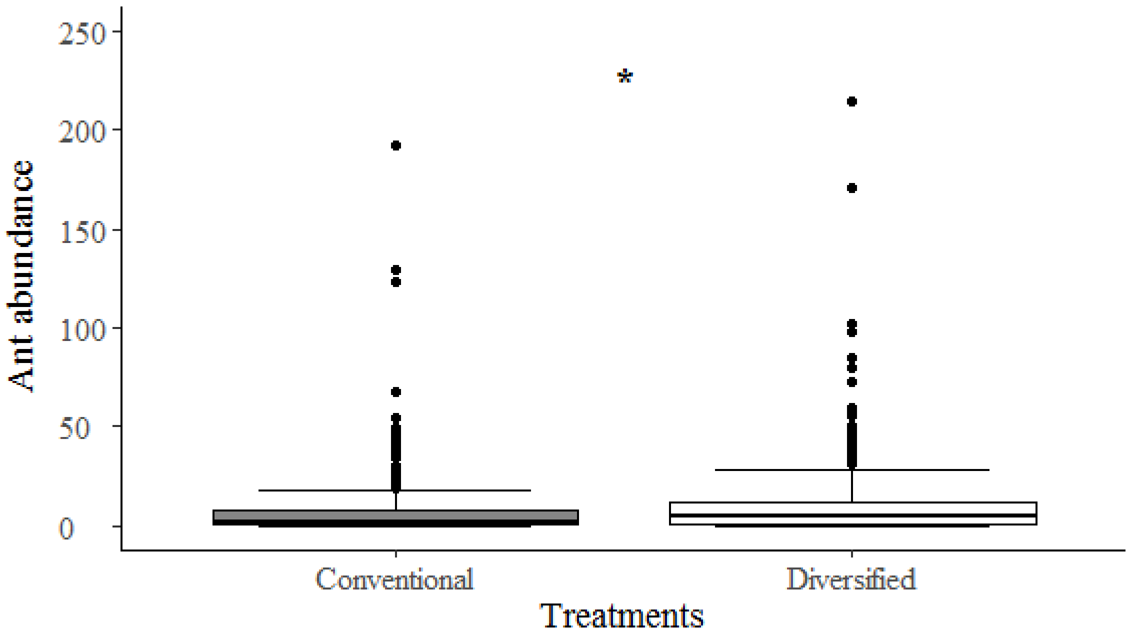

Figure 6 Box plot of the abundance of predatory ants in a conventional (gray box) and diversified (white box) coffee system ($\chi2$ = 18,134, df = 1, p <0.001). The asterisk represents the significant difference between the boxes.

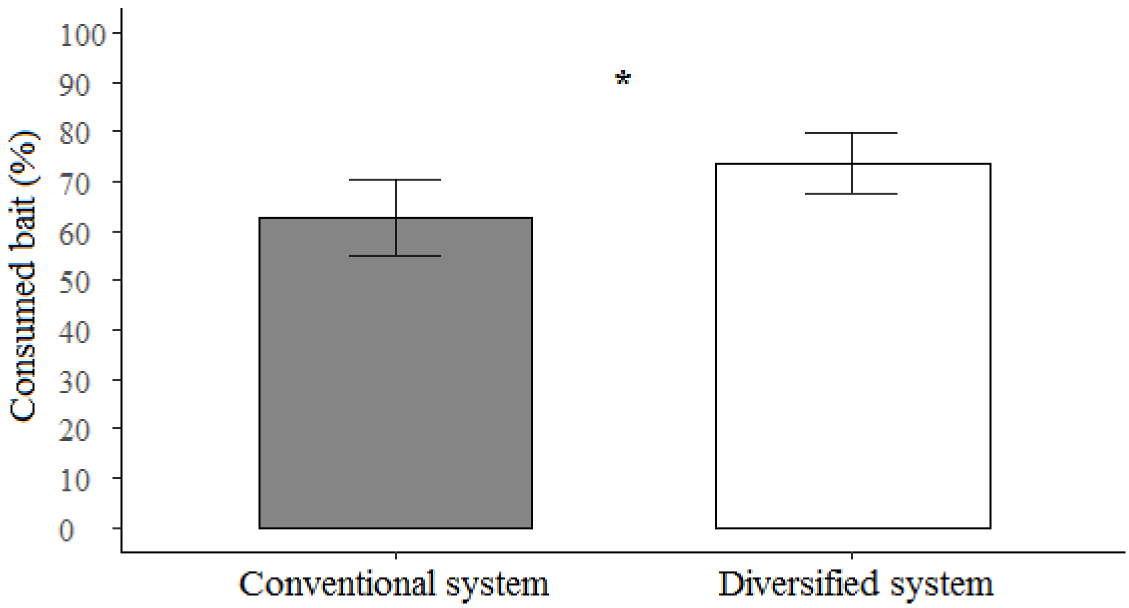

Figure 7 Proportion of consumed bait at two coffee system: conventional (gray bars) and diversified (white bars) ($\chi2$ = 8.668, df = 1, p <0.05). The asterisk represents the significant difference between the bars.

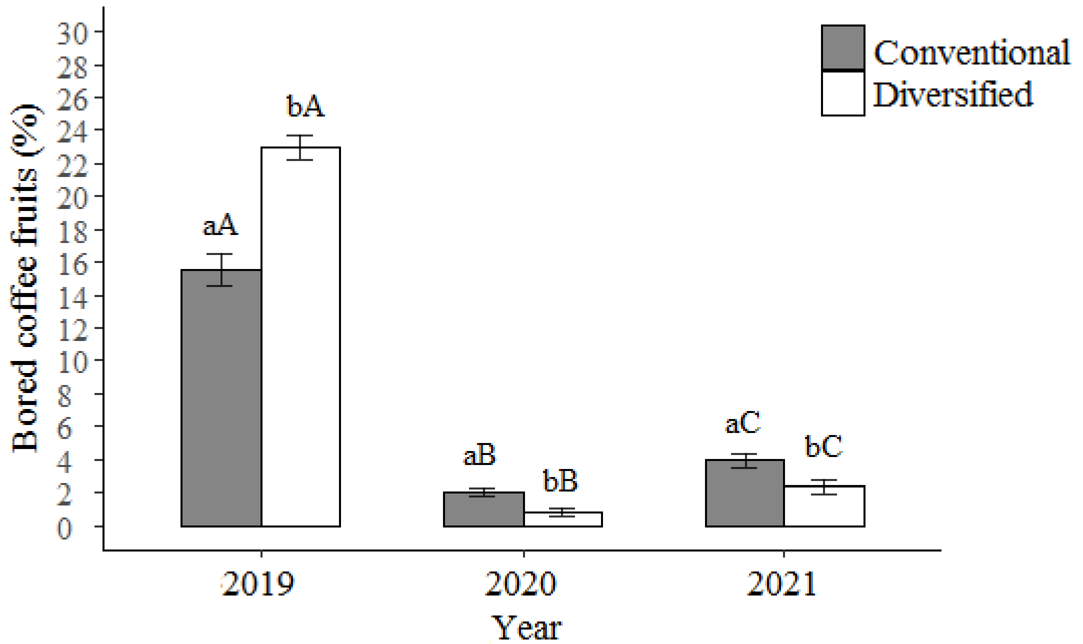

Figure 8 Average (± ep) of the proportion of bored berries in conventional (gray bar) and diversified (white bar) coffee systems, in the years 2019 (t = -12.704, p <0.001), 2020 (t = 5.330, p <0.001) and 2021 (t = 7.621, p <0.001). Lowercase letter differences in year and uppercase letter differences between years.

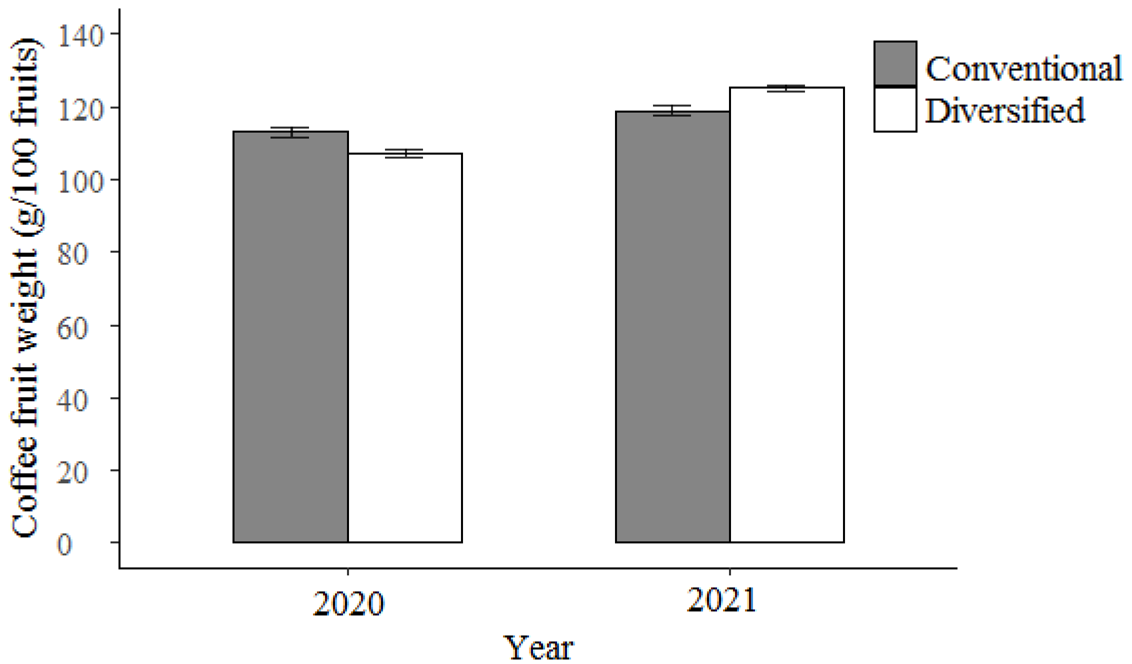

Figure 9 Average (± ep) of the coffee fruit weight (g/100 fruits) in conventional (gray bar) and diversified (white bar) coffee systems, in the 2020 and 2021 ($\chi 2$ = 0.856, df = 1, p =0.354).

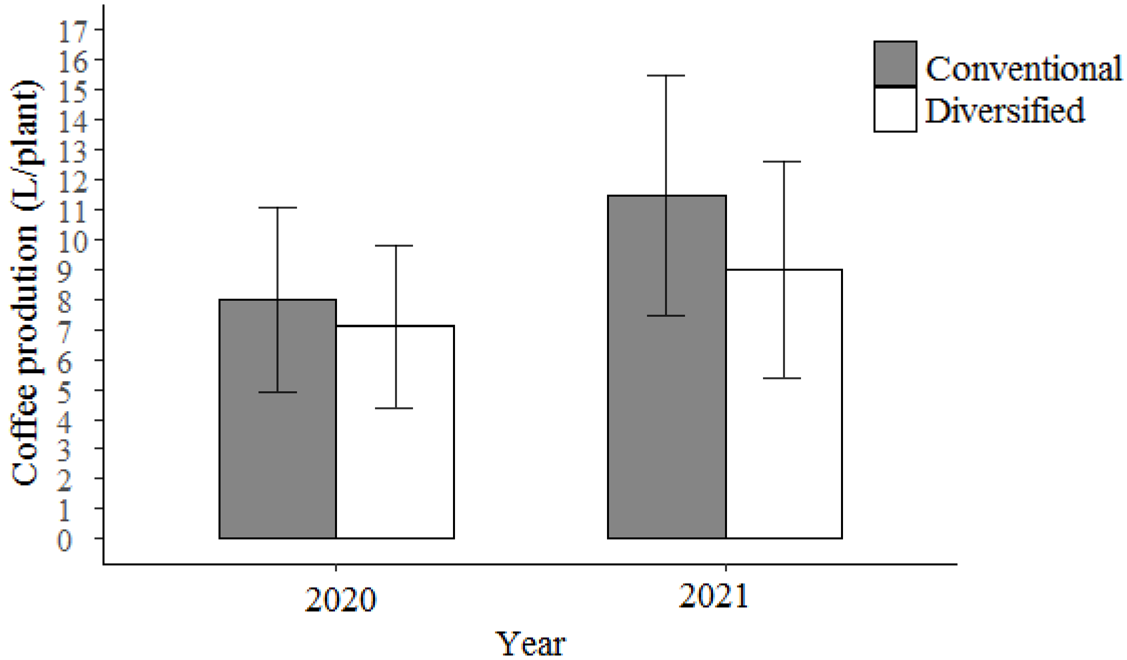

Figure 10 Average (± ep) of the coffee production (L/plant) in conventional (gray bar) and diversified (white bar) coffee systems, in the years 2020 and 2021 ($\chi 2 = 0.962$, df = 1, p =0.326).

Supplementary material

Appendix 1. Plant Nutrition (soil fertilization, coffee husk compost and foliar fertilization) in the plots with diversified and conventional coffee systems in the Experimental Research Station of EPAMIG, Patrocínio/MG, Brazil.

Soil fertilization

Date	Diversified coffee system	Conventional coffee system
Dec 2018	Urea (750 kg/ha)	Urea (750 kg/ha)
Feb 2019	NPK 20-05-20 (500 kg/ha)	NPK 20-05-20 (500 kg/ha)
Oct 2019	Limestone (1.5 t/ha); Gypsum (750 kg/ha); Simple superphosphate (500 kg/ha)	Limestone (1.5 t/ha); Gypsum (750 kg/ha); Simple superphosphate (500 kg/ha)
Nov 2019	Urea (300 kg/ha)	Urea (300 kg/ha)
Jan 2019	NPK 20-05-20 (500 kg/ha)	NPK 20-05-20 (500 kg/ha)
Mar 2020	NPK 20-00-20 (400 kg/ha)	NPK 20-00-20 (400 kg/ha)
Nov 2020	NPK 20-05-20 (500 kg/ha)	NPK 20-05-20 (500 kg/ha)
Dez 2020	Simple superphosphate (500 kg/ha)	Simple superphosphate (500 kg/ha)
Fev 2021	NPK 20-05-20 (500 kg/ha)	NPK 20-05-20 (500 kg/ha)

Coffee husk compost

Date	Diversified coffee system	Conventional coffee system
Dec 2018	5 kg/linear meter	5 kg/linear meter

Foliar fertilization

<table>
<tr><th>Date</th><th>Diversified coffee system</th><th>Conventional coffee system</th></tr>
<tr><td>Dec 2018</td><td rowspan="3">Potassium (10%), magnesium (2%), sulfur (8.26%), boron (6%), manganese (8%), molybdenum (0.10%) and zinc (3%) (5kg/ha)</td><td rowspan="2">Potassium (10%), magnesium (2%), sulfur (8.26%), boron (6%), manganese (8%), molybdenum (0.10%) and zinc (3%) (5kg/ha)</td></tr>
<tr><td>Feb 2019</td></tr>
<tr><td>Dec 2019</td><td>-----</td></tr>
<tr><td>Feb 2020</td><td>-----</td><td>Potassium (10%), magnesium (2%), sulfur (8.26%), boron (6%), manganese (8%), molybdenum (0.10%) and zinc (3%) (5kg/ha)</td></tr>
<tr><td>Mar 2020</td><td>Potassium (10%), magnesium (2%), sulfur (8.26%), boron (6%), manganese (8%), molybdenum (0.10%) and zinc (3%) (5kg/ha)</td><td>-----</td></tr>
<tr><td>Jan 2021</td><td rowspan="2">Nitrogen (10%), boron (1%), manganese (4%), copper (0.5%) and zinc (6%) (2 l/ha)</td><td rowspan="2">Nitrogen (10%), boron (1%), manganese (4%), copper (0.5%) and zinc (6%) (2 l/ha)</td></tr>
<tr><td>Abr 2021</td></tr>
</table>

Appendix 2. Pest and disease management in the plots with diversified and conventional coffee systems in the Experimental Research Station of EPAMIG, Patrocínio/MG, Brazil.

Insecticide

Date	Diversified coffee system	Conventional coffee system
Feb 2019	-----	Curbix® (2.5 l/ha)
Feb 2020		
Jan 2021		
Abr 2021		

Insecticide /Acaricide

Date	Diversified coffee system	Conventional coffee system
Dec 2018	-----	Ethiprole: phenylpyrazole (0.4 l/ha)
Feb 2019		
Feb 2020		
Jan 2021		Abamectin: avermectin (0.4 l/ha)
Abr 2021		

Fungicide

Date	Diversified coffee system	Conventional coffee system
Dec 2018	-----	Pyraclostrobin: strobilurin + epoxiconazole: triazole (1.5 l/ha) Copper hydroxide: inorganic (1.5 kg/ha)
Feb 2019		
Dec 2019	Copper hydroxide: inorganic (1.5 kg/ha)	-----
Feb 2020	-----	Pyraclostrobin: strobilurin + epoxiconazole: triazole (1.5 l/ha) Copper hydroxide: inorganic (1.5 kg/ha)
Mar 2020	Copper hydroxide: inorganic (1.5 kg/ha)	-----
Jan 2021	Copper hydroxide: inorganic (1.75 kg/ha)	Pyraclostrobin: strobilurin + epoxiconazole: triazole (1.5 l/ha) Copper hydroxide: inorganic (1.75 kg/ha) Boscalida: anilida (0.15 kg/ha)
Abr 2021		

Insecticide/Fungicide

Date	Diversified coffee system	Conventional coffee system
Dec 2019	-----	Thiamethoxam: neonicotinoid + cyproconazole: triazole (1 kg/ha)
Dec 2020		

Sodium hypochlorite

Date	Diversified coffee system	Conventional coffee system
Dec 2019	Solution 1%	-----
Mar 2020		
Jan 2021		
Abr 2021		

All the pesticides were applied with adjuvant mineral oil Agefix® (0.5%).

Appendix 3. Non-crop management in the plots with diversified and conventional coffee systems in the Experimental Research Station of EPAMIG Patrocínio/MG, Brazil.

Herbicide [1]

Date	Diversified coffee system	Conventional coffee system
May 2019	-----	Glyphosate: substituted glycine (1 kg/ha) 2,4-dichlorophenoxy: aryloxy alkanoic acid (1 l/ha)
Nov 2019		
May 2020		

Non-crop plants mechanic suppression

Date	Diversified coffee system	Conventional coffee system
May 2019	Suppressed up to 10 cm	Suppressed up to 10 cm
Jan 2020	Suppressed up to 50 cm	
Mar 2020		
Jun 2020	Suppressed up to 10 cm	
Dez 2020	Suppressed up to 50 cm	
Mar 2021		

Non-crop plants manual suppression [2]

Date	Diversified coffee system	Conventional coffee system
Mar 2019	Done	Done
Apr 2019		
Sep 2019		
Mar 2020		
Apr 2020		
May 2021		

[1] All the pesticides were applied with adjuvant mineral oil Agefix® (0.5%). [2] Manual suppression uses hoe around the diversified plants and in the flaws in the coffee row.

Appendix 4. Cost of pesticides in conventional coffee systems in the Experimental Research Station of EPAMIG Patrocínio/MG, Brazil.

Pesticides	Quantity/ha /application	Price (R$)	Cost/ha/ application (R$)	Application/ year	Manpower /ha/ application(R $)
Insecticides and Acaricides					
Abamectin: avermectin	0.4 L	36.00/L	14.40	2	-
Ethiprole: phenylpyrazole	2.5 L	123.00/L	307.50	2	-
Fungicides					
Pyraclostrobin: strobilurin + epoxiconazole: triazole	1.5 L	68.00/L	108.00	1	-
Boscalida: anilida	0.15 Kg	95.00/150g	95.00	1	-
Insecticide/Fungicide					
Thiamethoxam: neonicotinoid + cyproconazole: triazole	1 kg	370.00/Kg	370.00	1	90.00
Herbicides					
Glyphosate: substituted glycine	1 kg	30.00/kg	30.00	2	90.00
2,4-dichlorophenoxy: aryloxy alkanoic acid	1 L	28.00/L	28.00	2	
Total			1332.80		270.00

GENERAL CONCLUSION

The green lacewing *Chrysoperla externa* is reported for the first time as a predator of the coffee berry borer (CBB) *Hypothenemus hampei*. Therefore, we add a new predator to the list of species of CBB natural enemies.

Two species of green lacewing *C. externa* and *Ceraeochrysa cubana* are capable of preying on CBB inside the berries. However, *C. cubana* has a limitation in accessing the galleries as it is a trash-carrying larva. In addition, larvae of *C. externa* prey on CBB adults and limited their colonization of coffee berries.

The use of strategic diversification with *Inga edulis*, *Senna macranthera*, *Varronia currasavica* and non-crop plants, with absence of pesticides, increases the richness and abundance of predatory ants, decreasing CBB infestation, without altering coffee yield.