UNIVERSIDADE FEDERAL DE VIÇOSA

ELEM FIALHO MARTINS

CONSERVATION BIOLOGICAL CONTROL OF COFFEE LEAF MINER: ROLE OF GREEN LACEWINGS AND PARASITOIDS

VIÇOSA - MINAS GERAIS
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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: André Lage Perez
Jason M. Schmidt

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Assentimento:

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Madelaine Venzon
Orientadora

À mulher que me faz ter coragem todos os dias, minha mãe Selma

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À minha melhor companhia e eterno amor, meu marido André

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À toda família Martins e amigos

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(Tocando em frente - Almir Sater e Renato Teixeira)

RESUMO

MARTINS, Elem Fialho, D.Sc., Universidade Federal de Viçosa, outubro de 2021. **Controle biológico conservativo do bicho-mineiro do café: o papel de crisopideos e parasitoides.** Orientadora: Madelaine Venzon. Coorientadores: André Lage Perez e Jason M. Schmidt.

O bicho-mineiro do cafeeiro, *Leucoptera coffeella,* é uma praga-chave do café na região Neotropical. As larvas se alimentam do parênquima entre as superfícies das folhas do café, diminuindo a taxa de fotossintética, o que causa perdas significativas na produtividade e na longevidade das lavouras cafeeiras. Inimigos naturais como vespas, formigas, parasitoides e crisopideos estão envolvidos no controle biológico natural do bicho-mineiro. Porém, as populações desses inimigos naturais normalmente não são suficientes para diminuir ou manter as populações do bicho-mineiro abaixo dos níveis de controle em monoculturas de café, devido à dependência de recursos, como pólen e néctar que são escassos nos sistemas convencionais. Nesse sentido, a associação de outras nos plantios de café pode representar uma estratégia efetiva para incrementar a população de inimigos naturais na área, através do fornecimento de alimentos alternativos e de refúgio. Neste trabalho, investigou-se e a diversificação de lavouras de café (*Coffea arabica*) com *Inga edulis* "ingá", *Varronia curassavica* "erva-baleeira", *Senna macranthera* "fedegoso" e plantas espontâneas favorecem o controle biológico do bicho-mineiro por ação de predadores e parasitoides Essas plantas foram escolhidas por fornecerem recursos como néctar e pólen constantemente, abrigo e sítios de oviposição e acasalamento para inimigos naturais do bicho-mineiro, através dos nectários extraflorais (*I. edulis* e *S. macranthera*) e/ou por apresentar florescimento constante (*V. curassavica*). Primeiramente, nós realizamos experimentos de laboratório para avaliar se os estágios imaturos do crisopideo bicho-lixeiro *Ceraeochrysa cubana* predam os estágios imaturos do bicho-mineiro do café (Capítulo I). Larvas de *C. cubana* se alimentaram com sucesso de ovos e pupas do bicho-mineiro, sendo esse o primeiro registro de um crisopideo como predador de ovos do bicho-mineiro, adicionando essa espécie à lista de predadores desta praga-chave. Posteriormente, investigou-se se *V. curassavica* fornece seletivamente recursos para *C. cubana,* sem beneficiar adultos do bicho-mineiro (Capítulo II). Larvas do predador sobreviveram por mais tempo na presença das inflorescências de *V. curassavica,* já as fêmeas do bicho-mineiro tiveram redução da taxa de crescimento na presença das inflorescências. No campo, avaliou-se os visitantes das plantas inseridas e se a diversificação estratégica com *I. edulis*, *S. macranthera* e *V. curassavica* em lavouras de café, com manutenção de plantas espontâneas e sem aplicações de agrotóxicos influencia a abundância e a riqueza de crisopideos e vespas, a taxa de parasitismo, de predação e o controle do bicho-mineiro comparado com sistemas convencionais de café em monocultura (Capítulo III). As formigas são os visitantes mais abundantes de *I. edulis*, *S. macranthera* e *V. curassavica*. Não houve diferença na abundância de crisopideos e de vespas entre os sistemas, no entanto, no sistema diversificado houve incremento das populações de parasitoides. Apesar da maior taxa de parasitismo em sistemas diversificados, não houve diferença na infestação do bicho-mineiro em comparação aos sistemas convencionais em 2019 e 2021, e foi maior em 2020. Entretanto, em nenhum dos sistemas a população de bicho-mineiro atingiram o nível de controle. Nosso estudo demonstra por meio de experimentos de laboratório e de campo que é possível aprimorar o controle biológico do bicho-mineiro com estratégias seguras ao meio ambiente e ao homem, além de manter a biodiversidade nos agroecossistemas cafeeiros.

Palavras-chave: *Leucoptera coffeella*. Controle biológico conservativo. *Ceraeochrysa cubana*, *Varronia curassavica*

ABSTRACT

MARTINS, Elem Fialho, D.Sc., Universidade Federal de Viçosa, October, 2021. **Conservation biological control of coffee leaf miner: role of green lacewings and parasitoids.** Adviser: Madelaine Venzon. Co-advisers: André Lage Perez and Jason M. Schmidt.

Coffee leaf miner, *Leucoptera coffeella*, is a key coffee pest in the Neotropics. The larvae feed on the parenchyma between surfaces of coffee leaves causing decrease of the photosynthesis rate leading to significant losses in yield and in the longevity of coffee plant. Several natural enemies are involved in coffee leaf mine biological control, such as wasps, ants, parasitoids and green lacewings. However, normally their populations are not enough to decrease or to maintain the coffee leaf mine populations below threshold levels in coffee monocultures, due to their dependence on resources, such as pollen and nectar, which are scarce in conventional systems. The association of plant species to coffee crops might represent an effective strategy through the provision of alternative food and refuge for natural enemies. I investigate here whether the diversification of *Coffea arabica* crops with I*nga edulis* "erva-baleeira", *Varronia curassavica* "inga", *Senna macranthera* "fedegoso" and non-crop plants favors the biological control of coffee leaf miner by its predators and parasitoids. These plants can provide resources such as nectar and pollen constantly, shelter and oviposition and mating sites for natural enemies with either extrafloral nectaries (*I. edulis* and *S. macranthera*) and/or inflorescences (*V. curassavica*). Firstly, we performed laboratory experiments to evaluate whether the immature stages of the green lacewing *Ceraeochrysa cubana* are able to prey on the immature stages of coffee leaf miner (Chapter I). Larvae of *C. cubana* successfully prey on eggs and pupae of coffee leaf miner, being the first report about green lacewing predation on the pest eggs. We also investigate whether *V. curassavica* provides selective resources to *C. cubana* without benefiting coffee leaf miner adults (Chapter II). Larvae of *C. cubana* survived longer in the presence of *V. curassavica* inflorescences, and decrease the population growth rate of the pest. In the field, we evaluated the visitors of the inserted plants and whether the strategic diversification with *I. edulis*, *S. macranthera* and *V. curassavica* added to non-crop areas under management of no pesticide use influence the abundance and richness of predatory green lacewings and wasps, increase the parasitism and control coffee leaf miner compared to conventional coffee systems (Chapter III). I found that ants are the most abundant visiting insects of *I. edulis*, *S. macranthera* and *V. curassavica*. In addition, I found that although there was no significant difference in the abundance of green lacewings and wasps between systems, parasitism rate was higher in the diversified. Despite that, there was no difference in the pest infestation comparing to conventional coffee systems in 2019 and 2021, but in 2020 it was higher in the diversified. However, CLM populations did not reach threshold in any system. Our study demonstrates through laboratory and field experiments that it is possible to improve the biological control of coffee leaf miner with safe strategies to environment and humans, in addition to maintaining biodiversity in coffee agroecosystems.

Keywords: *Leucoptera cofeella*. Conservation biological control. *Ceraeochrysa cubana*, *Varronia curassavica*

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GENERAL INTRODUCTION

The Brazil is the largest producer and exporter of coffee of the world (Faostat, 2019). However, several factors can negatively impact the coffee production, as the attack by coffee leaf miner (CLM), *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae), a key coffee pest in the Neotropics (Souza et al., 1998; Pereira et al., 2007; Fernandes et al., 2009; Pantoja-Gomez et al., 2019). The pest native to Africa was reported by the first time in America in 1842, in 1850 in Brazil and later to most American coffee-producing countries (Green, 1984). Larvae CLM feed on the cells of palisade parenchyma, decreasing photosynthesis of coffee plant (Souza et al., 1998; Pereira et al., 2007; Giraldo-Jaramillo et al., 2019). At high population levels, CLM may cause defoliation up to 70% and reduce coffee yields by 50% (Reis and Souza, 1996). The pest often attains high population levels in unshaded, dry and hot areas, conditions often found in many coffee production regions in Brazil (Reis et al., 2002; Leite et al., 2020).

Pesticides are the most common measure use in attempt to control CLM in heavy infestations (Leite et al., 2020). However, pest resistance evolution, outbreaks of secondary pests, and loss of beneficials are some of the concerns associated with reliance on pesticide applications (Fragoso et al., 2003; Pereira et al., 2007; Guedes et al., 2016, 2017; Leite et al., 2020, 2021), which highlight the need for alternative management tactics, and the identification of natural control agents. Several predator and parasitoid species are associated to CLM (Pereira et al., 2007; Lomelí Flores et al., 2009, 2010; Fernandes et al., 2009). The most common natural enemies are wasps, ants and hymenopteran parasitoids (Pereira et al., 2007; De la Mora et al., 2008; Lomelí-Flores et al., 2009; Rezende et al., 2014; Androcioli et al., 2018). To increase the naturally occurring biological control of CLM in coffee crops is necessary to join strategies that attract and to maintain natural enemies in the crop, such as plant diversification in coffee crops (Rezende et al., 2014, 2021; Rosado et al., 2021; Venzon, 2021). The plants associated to the main crop should provide essential and supplementary resources to natural enemies, including food, shelter and oviposition sites (Landis et al., 2000; Gurr et al., 2003; Venzon et al., 2006; Tscharntke et al., 2008). Thus, the choice of these plants needs to be strategic to ensure the provision of selective resources to natural enemies and not to the pests (Venzon et al., 2006; Lavandero et al., 2006; Venzon and Sujii, 2009; Chen et al., 2020). Therefore, knowing the interactions between the associated plants and their visiting insects is decisive for the proper employment of the conservation biological control strategies (Venzon et al., 2001). Additionally, the introduced plants should require minimal cultivation and not compete by nutrients with the coffee plants (Venzon et al., 2006; Lavandero et al., 2006; Venzon and Sujii, 2009; Souza et al., 2010; Venzon et al., 2011).

In this thesis, I investigate whether the diversification of *Coffea arabica* crops with strategic selected plants favors the biological control of CLM by predators and parasitoids. I associated *Inga edulis*, *Varronia curassavica*, *Senna macranthera* and non-crop plants to coffee plants. They were selected based on their provision of food resources to natural enemies of CLM through extrafloral nectaries (*I. edulis* and *S. macranthera*) and flowers (*V. curassavica*). I used conventional coffee plots (monoculture) as control. Since beginning of field evaluations, I frequently found Chrysopidae adults on *V. curassavica*. Therefore, I also performed laboratory experiments to evaluate whether the immature stages of Chrysopidae preying on the immature stages of CLM (Chapter I). I also investigate whether *V. curassavica* provides selective resources to a Chrysopidae species without benefiting CLM adults. Finally, in Chapter III, I evaluated whether the strategic diversification with *I. edulis*, *S. macranthera* and *V. curassavica* in coffee crops increases the biological control of CLM by action of green lacewings, wasps and parasitoids. For this, I evaluated the floral and extrafloral nectary visitors of the associated plants, compared the abundance and richness of green lacewings and wasps on coffee, CLM parasitism rate and CLM infestation rate between diversified and conventional coffee systems. In this sense, this study demonstrates through field experiment combined with laboratory experiments that it is possible to join the efficient biological control of CLM, with more secure to the environmental and human, in addition to maintaining biodiversity in coffee agroecosystems. Additionally, this study can be used as a basis to new research’s that involves of coffee diversification and in the same time can be used by farmers aiming the management of CLM in coffee crops.

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Is *Ceraeochrysa cubana* a coffee leaf miner predator?

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HIGHLIGHTS

- *Ceraeochrysa cubana* larvae successfully prey on coffee leaf miner (CLM) eggs.
- Second and third instar of *C. cubana* larvae prey on CLM larvae and pupae.
- The predator *C. cubana* is a candidate for biocontrol programs of CLM.

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ABSTRACT

Coffee leaf miner (CLM), *Leucoptera coffeella,* is a key pest of unshaded coffee in hot, dry neotropical production areas. Control is hampered by CLM mining behavior that reduces biological control and pesticide efficacy. Therefore, finding natural enemies that can efficiently control CLM could improve conservation and augmentative biological control practices, and potentially reduce reliance on increasingly ineffective insecticide applications. The green lacewing *Ceraeochrysa cubana* is a generalist predator often found in coffee crops in Brazil, but there is little information regarding its potential contribution to CLM control. We investigated whether *C. cubana* preys on immature stages of CLM and whether predation levels vary according to life stage of *C. cubana* or CLM. First, second and third instar *C. cubana* readily consumed CLM eggs, but once inside mines, few CLM larvae were consumed. Second and third instar *C. cubana* successfully preyed on CLM pupae, but first instars did not. Thus, all three instars of *C. cubana* were able to prey on at least one immature stage of CLM, with potential to impede mine formation and adult emergence.

1. Introduction

The coffee leaf miner (CLM), *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae), is a key coffee pest in the Neotropics (Souza et al., 1998; Pereira et al., 2007; Fernandes et al., 2009; Pantoja-Gomez et al., 2019). CLM females lay eggs on the adaxial leaf surface of coffee plants and, after hatching (3 to 21 days), the larvae feed on the cells of palisade parenchyma (Souza et al., 1998; Pereira et al., 2007; Giraldo-Jaramillo et al., 2019). At high population levels, CLM may cause defoliation up to 70%, which decreases photosynthesis and can reduce coffee yields by 50% (Reis and Souza, 1996). *Leucoptera coffeella* often attains high population levels in unshaded, dry and hot areas, conditions often found in many coffee production regions in Brazil (Reis et al., 2002; Leite et al., 2020). Pesticides are the most common measure for controlling CLM in heavy infestations, and applications can be as many as 20 per year (Leite et al., 2020). Of course, there are many concerns associated with reliance on pesticide applications, including pest resistance evolution, outbreaks of secondary pests, and loss of beneficials (Fragoso et al., 2003; Pereira et al., 2007; Guedes et al., 2016, 2017; Leite et al., 2020, 2021), which highlight the need for alternative management tactics, and the identification of natural control agents.

Many natural enemy species are reported to prey on, or to parasitize, CLM when conditions are favorable (Pereira et al., 2007; Lomelí-Flores et al., 2009, 2010; Fernandes et al., 2009). The most common predators

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are wasps and ants (Pereira et al., 2007; De la Mora et al., 2008; Lomelí-Flores et al., 2009; Androcioli et al., 2018), and hymenopteran parasitoids, predominately in the families Braconidae and Eulophidae (Pereira et al., 2007; Lomelí-Flores et al., 2009; Rezende et al., 2014). Although green lacewings (Neuroptera: Chrysopidae) are often found in coffee crops (Pappas et al., 2011; Ribeiro et al., 2014; Barbosa et al., 2019; Martins et al., 2019), their role in biological control of CLM requires further study. Chrysopidae larvae are voracious and active foragers that prey mainly on soft-bodied arthropods such as scales, aphids, small caterpillars, insect eggs and mites (Canard and Principi, 1984; Albuquerque et al., 1994; Tauber et al., 2009; Freitas and Penny, 2012). Adult lacewings commonly feed on pollen, nectar and honeydew and have a high reproductive potential (Venzon and Carvalho, 1992; Venzon et. al., 2006; Tauber et al., 2009). In a laboratory study, Ecole et al. (2002) observed predation by third instar *Chrysoperla externa* (Hagen) on CLM larvae, pre-pupae and pupae. Despite the often-high abundance of green lacewings in coffee crops (Ribeiro et al., 2014; Martins et al., 2019), to our knowledge, no study has yet documented the potential of green lacewings for CLM control. If shown to be effective, mass reared chrysopids would be commercially available for release as either eggs or larvae (Souza and Bezerra, 2019).

We investigated predation of CLM by a green lacewing that commonly occurs in the coffee agroecosystems of Brazil, *Ceraeochrysa cubana* (Hagen) (Ribeiro et al., 2014; Martins et al., 2019). The larvae of *C. cubana* are trash-carriers; they attach prey remains and other debris to the hooked setae that cover their backs, thus obtaining physical protection and camouflage (Canard and Duelli, 1984). Here, we used a series of laboratory studies to examine whether *C. cubana* preys on CLM, and how predation varies according to pest and predator developmental stages. Our aim was to provide information that might improve either the conservation or the augmentation of green lacewings for CLM control.

2. Materials and methods

2.1. Rearing of coffee leaf miner

We established a colony of CLM in the Laboratory of Entomology at Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG) using material field-collected from CLM infested leaves from plants in an experimental coffee crop located at the Campus of the Federal University of Viçosa (UFV), Viçosa, MG, Brazil. The colony was maintained under a 12 h photoperiod regime, at 23 ± 1 °C and 70 ± 2% RH. To keep the leaves hydrated, we inserted their petioles into foam sections soaked in water and placed them inside plastic boxes (20 × 10 cm), which were kept inside transparent acrylic cages (40 × 40 × 40 cm). Daily, we removed newly emerged adults and transferred them to new cages with clean coffee leaves, to maintenance the rearing (Adapted from Reis Jr. et al., 2000).

2.2. Rearing of green lacewing

Ceraeochrysa cubana were reared in the laboratory under the same climate conditions as described above. We maintain the genetic diversity of our laboratory colony of *C. cubana* via the periodic introduction of wild individuals from the field. We sent specimens from our colony to Dr. Renildo Ismael Félix Costa to confirm their taxonomic identification. We reared the adult lacewings in cylindrical PVC cages (10 cm in diameter and 20 cm in height), and fed them with a diet based on yeast and honey (1:1), which was offered on a parafilm strip hung inside the cage (Venzon et al., 2006). We provided water on a piece of soaked cotton that was placed inside a 10 mL vial. Food and water were replaced twice per week. We collected eggs of *C. cubana* from the cages by cutting their pedicels and transferred them to glass tubes (2.5 × 8.5 cm). We fed the newly emerged larvae with eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) until they reached the pupal stage.

2.3. Predation of CLM eggs by C. cubana

2.3.1. Egg predation on leaves

We evaluated the potential of first, second and third instar *C. cubana* larvae to prey on CLM eggs. We used newly-emerged first instar larvae, and second and third instars reared on *E. kuehniella* eggs. All larvae were starved for a period of 24 h before testing. We offered each predator larvae a coffee leaf with a single two-day old CLM egg. To obtain the eggs, we collected healthy coffee leaves from an experimental coffee crop at UFV, washed them in water, and checked the leaves under a microscope to ensure that there were no other arthropods or eggs. After checking the leaves, we placed them inside transparent acrylic cages (40 × 40 × 40 cm) in the presence of several CLM adults, to allow the females lay eggs on them. After 48 h, we removed the leaves and removed all laid eggs except for one. Additionally, the domatia of each coffee leaf were covered with tape, to prevent them harboring any other predators, as some arthropods will use coffee domatia as a refuge (Matos et al., 2006). This procedure was done for all experiments. We inserted the petiole of each leaf with the CLM egg in a plastic container (3 mL) with water to maintain leaf turgidity. After that, we placed the leaves individually inside plastic pots (500 mL) and we added one *C. cubana* larvae to each pot. We sealed the pots with PVC film and after 24 h we removed the predator. As a control, we used a coffee leaf with a CLM egg inside the plastic pot but without predator, which enabled us to compare the mortality of eggs in the presence and in the absence of the predator. In total, we completed 30 replications for each one of the three predator instars and 30 for the control. Due to difficulties in identifying the predated egg from the intact ones, predation was evaluated indirectly, considering the intact as those that originated mines (Venzon et al., 2005). We thoroughly inspected leaves over 20 days after predator removal to record mine formation, as it is the maximum egg hatching period for coffee leaf miner (Souza et al., 1998). During this time, leaves were kept with their petioles inserted in water to ensure their suitability to CLM (Pereira et al., 2007; Rezende et al., 2014).

2.3.2. Egg predation on coffee seedlings

In order to assess CLM egg predation under more realistic conditions, we carried out an experiment using potted coffee seedlings with two true leaves, so that predators had to search for prey on a plant. Also, in this experiment, we did not limit prey to one CLM egg. We reared newly emerged *C. cubana* larvae on *E. kuehniella* eggs until they had reached the second instar, as second instar larvae are more visible than first instars and suffer less mortality when manipulated. All individuals were starved for a period of 24 h prior to testing. Coffee seedlings (n = 30 per treatment) were cleaned and held in the laboratory at 25 ± 2 °C, 70 ± 2% RH, and a 12:12 (L:D) photoperiod. We thoroughly inspected each seedling to ensure that there were no other arthropods or eggs. A transparent plastic cylinder (32 × 12 cm), with two rectangular openings (10 × 6 cm) covered with voile cloth, was used to protect each seedling. The cylinder was inserted in the soil of the pot and a plastic disc (15 cm diam) was placed over the soil to prevent insects from reaching the soil. Three CLM adults (two females and one male) were then placed on each plant and females were left to lay eggs for 48 h, after which we removed the adults and eggs, leaving five eggs per seedling. One second instar *C. cubana* larvae was then placed inside each cylinder and it was sealed. After 48 h, we removed the predators and we observed the CLM eggs under a microscope. As a control treatment, we set up coffee seedlings with five CLM eggs, but without the predator. The frequency of mine formation was tallied over 20 days as an indication of no predation, (as in 2.2.1), as this is the maximum incubation period for coffee leaf miner eggs (Souza et al., 1998).

2.4. Predation of CLM larvae by C. cubana

We evaluated the potential for first, second and third instar larvae of *C. cubana* to prey on CLM larvae. Predator larvae were obtained as explained above and each was offered a coffee leaf with one CLM in a mine, obtained from an experimental coffee plantation at UFV. We examined each leaf under a microscope to ensure that the CLM larva was alive, washed it in water, and cleaned it with wet cotton to remove any other insects or eggs. The domatia of each coffee leaf were covered with tape, as explained above (item 2.2.1) and then each leaf petiole was inserted into a plastic container (3 mL) of water to maintain leaf turgidity (Pereira et al., 2007). Leaves were then placed individually in plastic pots (500 mL) and one *C. cubana* larvae was added to each pot. We sealed the pots with PVC film and then removed the predator after 24 h. Thirty replications were conducted with each of the three instars, and 30 for the control. The control consisted of a coffee leaf with a CLM mine in a plastic pot without a *C. cubana* larvae. We inspected leaves under a microscope after 24 h and considered intact mines as evidence of no predation, and the presence of perforations in the mines and shrunken larvae as evidence of predation.

2.5. *Predation of CLM pupae by C. cubana*

We evaluated the potential of first, second and third instar larvae of *C. cubana* to prey on CLM pupae (n = 30 per treatment in all cases). Predator larvae used in the experiments were obtained as explained above and a coffee leaf with one CLM pupa was offered to each individual predator larva. To obtain these, we collected coffee leaves with pupae from an experimental coffee plantation at UFV and examined the leaves under a microscope to ensure that each pupa was alive. We then cut each leaf into a circle containing the pupa, placed each in a plastic pot (500 mL), and added one *C. cubana* larva. We sealed each pot with PVC film and removed the predator 24 h later. A leaf circle with a CLM pupa in a plastic pot without a predator served as a control. Again, predation was determined by examination of pupae under a microscope 24 h after predator removal.

2.6. *Data analyses*

For CLM egg predation, zero was recorded when no mine was formed (i.e., predation by *C.cubana*), versus one for successful mines (no predation). The resulting binary data were analyzed using generalized linear models (GLM) adjusted to a binomial distribution. Treatments were compared using the z-test within the GLM procedure, using control values as the intercept, allowing us to directly contrast the effect of *C. cubana* instar against *C. cubana* absence (Crawley, 2007). Data on predation on coffee seedlings were used to determine percentage egg mortality in the presence or absence *C. cubana* with a population of known number (n = 5). Data were analyzed using a GLM model adjusted for overdispersion correction with the quasi-binomial distribution. Percent egg mortality was analyzed using an independent *t*-test (Crawley, 2007). CLM larval and pupal mortality data were tallied as 'zero' (alive) and 'one' (dead), and analyzed using GLM adjusted with a binomial distribution. We also fixed our control as the intercept's model to estimate the differences in CLM larval and pupal mortality among treatments for different instars and compared them using the z-test within the GLM procedure. All analyzes were performed in software R version 4.0.2 (R Development Core Team, 2018).

3. Results

3.1. *Predation of CLM eggs*

Larvae of *C. cubana* pierced CLM eggs and sucked out their contents (Video 1). The percentage of mine formation on leaves was significantly lower in the presence of *C. cubana* than in its absence, for the first (z = 4.186; df = 3; p < 0.001), second (z = 4.364; df = 3; p < 0.001) and third instar larvae (z = 3.282; df = 3; p = 0.005) (Fig. 1). The presence of first, second and third instar *C. cubana* larvae reduced the percentage of mine formation by 70.0, 73.6 and 53.3%, respectively.

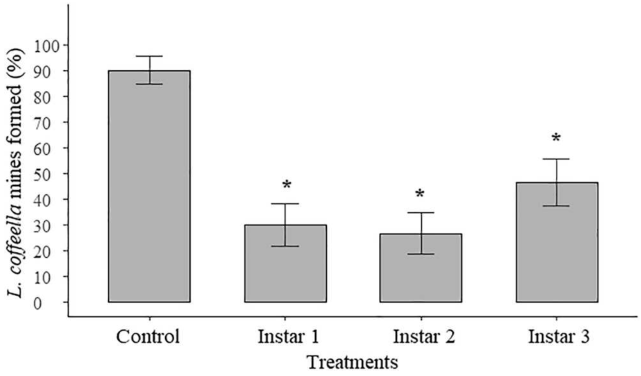

Fig. 1. Percentage of coffee leaf mine formation in the presence of *C. cubana* larvae of first (z = 4.186; df = 3; p = 0.0002), second (z = 4.364; df = 3; p = 0.0001) and third instar (z = 3.282; df = 3; p = 0.005), compared to the absence of *C. cubana* (control). Asterisks on the bars represent significant differences in relation to the control.

On coffee seedlings, the percentage of CLM egg mortality was significantly higher in the presence of predator larvae than in the predator absence (t = 5.92; df = 1; p < 0.001; Fig. 2). The presence of second instar larvae of *C. cubana* reduced mine formation by 82.6% on coffee seedlings.

3.2. *Predation of CLM larvae*

Mortality of CLM larvae did not differ whether first (z = 0.0; df = 3; p = 1.0), second (z = -0.005; df = 3; p = 1.0) or third (z = -0.006; df = 3; p = 1.0) instar *C. cubana* larvae were present or not (Fig. 3). Only four third instar larvae (13.3%) were able to prey on CLM larva. Direct observations indicated that larvae identified the mine with their mandibles, pierced the leaf tissue, and then preyed on them inside the mine (Video 2).

3.3. *Predation of CLM pupae*

CLM pupal mortality did not differ whether *C. cubana* first instar larvae were present of absent (z = -2.146; df = 3; p = 0.138), but pupal mortality was significantly higher in the presence of second (z = -4.682; df = 3; p < 0.0001) and third instars (z = -4.682; df = 3; p < 0.0001; Fig. 4). The presence of first, second and third instar of *C. cubana* larvae increased respectively in 26.7%, 96.7% and 96.7% the percentage mortality of CLM pupae inside the cocoon.

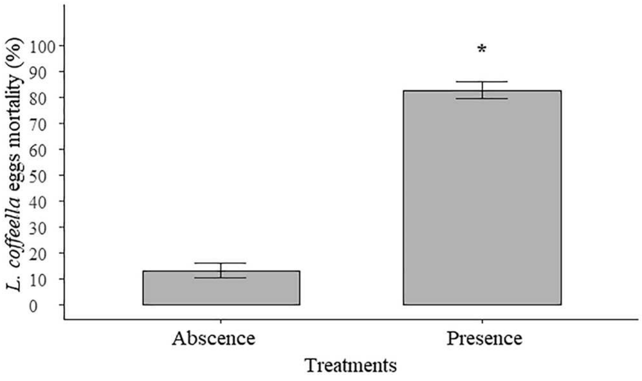

Fig. 2. Percentage of coffee leaf miner egg mortality in the presence of second instar larvae of *C. cubana* compared to the absence of *C. cubana* on coffee seedlings (t = 5.92; df = 1; p < 0.001). Asterisks on the bars represent significant differences in relation to the control.

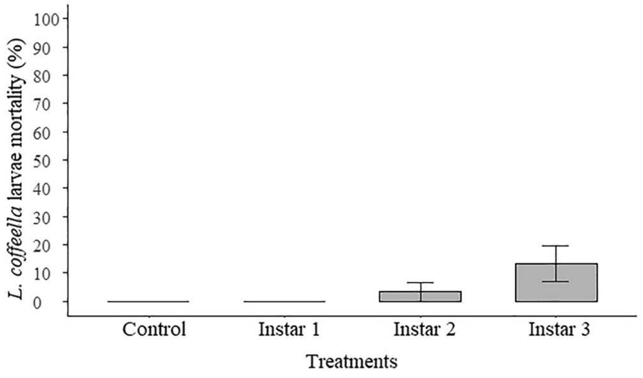

Fig. 3. Percentage of coffee leaf miner larvae mortality in the presence of larvae of first (z = 0.0; df = 3; p = 1.0), second (z = -0.005; df = 3; p = 1.0) and third instar of *C. cubana* (z = -0.006; df = 3; p = 1.0) compared to the absence of *C. cubana* (control).

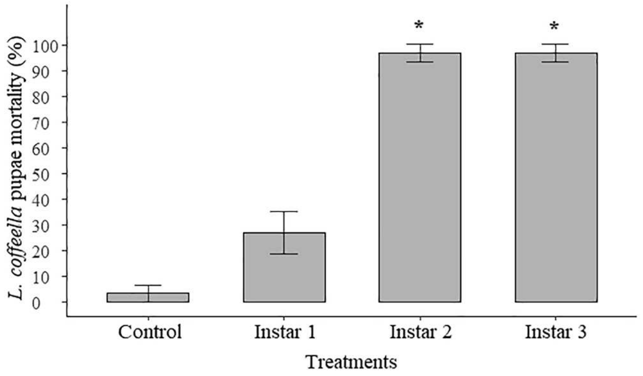

Fig. 4. Percentage of coffee leaf miner pupae mortality in the presence of larvae of first (z = -2.146; df = 3; p = 0.138), second (z = -4.682; df = 3; $p < 0.0001$) and third instar of *C. cubana* (z = -4.682; df = 3; $p < 0.0001$) compared to the absence of *C. cubana* (control). Asterisks on the bars represent significant differences in relation to the control.

4. Discussion

This study is the first to document predation of CLM eggs by a chrysopid species. We observed *C. cubana* larvae actively searching for eggs, finding and piercing them with their mandibles, and leaving behind shrunken, empty egg chorions. The only other study to examine chrysopid-CLM interactions reported that third instar *C. externa* larvae were able to recognize CLM eggs upon encounter, but did not prey on them (Ecole et al., 2002), although the authors did not describe the criteria used to determine predation. Because egg predation is difficult to distinguish, we used mine formation as an indirect measure of predation. Thus, we believe that predation by *C. externa* can occur, as both species are found on coffee crops and have similar feeding behavior, although this remains to be verified.

It is common to find all instars of Chrysopidae on coffee leaves and fruits, and *C. cubana* is among the most abundant species (Martins et al., 2019; Ribeiro et al., 2014). First instar *C. cubana* larvae are ready to prey on CLM eggs immediately following eclosion. We observed that second instar larvae searched actively for CLM eggs on coffee seedlings, and preyed on those they encountered. Most previous studies have focused on control of CLM larvae (Fragoso et al., 2003; Pereira et al., 2007; Leite et al., 2020), but our results suggest that the egg stage provides a window of opportunity for predation by *C. cubana*, an important observation because predation of eggs prevents any plant damage. Thus, predation of eggs by *C. cubana*, in combination with the actions of other predators such as ants (Lomelí-Flores et al, 2009), has the potential to contribute to biological control of this pest.

Once CLM larvae formed mines, they became largely immune to predation by first, second and third instar *C. cubana* larvae, despite occasional predation events by second and third instars (3% and 13.3%, respectively). We suspect that the leathery cuticle of coffee leaves could impair penetration of the leaf by chrysopid mandibles, thus preventing feeding (Ribeiro et al., 2007). Instars of *C. cubana* that successfully preyed on CLM larvae appeared use their mandibles to identify the mine, before piercing the mine and preying on the larva within. Observations under a stereomicroscope revealed that, after a *C. cubana* larva pierced the mine, the CLM larvae made twitching movements to try and avoid predation, suggesting an antipredator response to mine penetration. Further study of the behavior of CLM within mines in the presence of predators could better clarify these interactions.

Second and third instar of *C. cubana* were able to prey on CLM pupae within mines, preventing adult emergence, although first instar larvae did not. Possibly, the small, fragile mouthparts of first instar *C. cubana* larvae make it difficult for them to puncture the pupal integument. Ecole et al. (2002) reported that 50% of offered CLM pupae were preyed upon by third instar *C. externa*, but did not test other instars. Our results suggest that later instars of *C. cubana* are more effective at preying on CLM pupae than first instars.

CLM eggs are difficult to see with the naked eye due to their small size (0.3 mm), which makes direct observation of CLM predation by larvae of *C. cubana* on coffee leaves in the field an unlikely event. CLM pupae are usually found on the abaxial surface of coffee leaves, which could also reduce the probably predation events are directly observed in coffee fields. Our results indicate that *C. cubana*, and possibly other chrysopid species, can impair both the formation of CLM mines, and the emergence adult moths, thus potentially diminishing both leaf damage and pest population increase. Various predatory wasp species are also known to prey on coffee leaf miner larvae by tearing open mines and removing the larva (Pereira et al., 2007; Fernandes et al., 2009; Androcioli et al., 2018), thus providing complementary CLM mortality in coffee agroecosystems. Adults of *C. cubana* and other predators in coffee crops feed on pollen and sugary foods of plant origin, so their conservation in the coffee agroecosystem will depend to some extent on the availability of these resources, in addition to suitable microclimate conditions. Therefore, conservation biocontrol of CLM will benefit from plant diversity and the presence of non-crop plants, especially those providing nectar, pointing to the value of intercropping coffee with cover crops and trees (Venzon et al., 2006, 2019; Amaral et al., 2010; Rezende et al., 2014; Rosado et al., 2021). Also, mass rearing procedures for *C. cubana* are well developed (Venzon and Carvalho, 1992; Carvalho and Souza, 2000; Souza and Bezerra, 2019), raising the possibility of an augmentation approach. In summary, our results reveal that larval stages of *C. cubana* will prey on CLM eggs and pupae, and have the potential to contribute to biological control of this key coffee pest.

CRediT authorship contribution statement

Elem F. Martins: Conceptualization, Methodology, Investigation, Writing - original draft. **Mayara L. Franzin:** Methodology. **André L. Perez:** Formal analysis, Supervision. **Jason M. Schmidt:** Writing - review & editing, Supervision. **Madelaine Venzon:** Supervision, Project administration, Writing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2021.104691.

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Chapter II[1]

Opposing effects of a medicinal plant on predator and pest fitness

ABSTRACT

The introduction of different plant species in agricultural systems can improve the biological control of pests through provision of alternative resources to natural enemies. Non-prey food such as nectar, pollen, and alternative non-pest food are commonly cited as explaining the importance of promoting plant diversity in agricultural landscapes. Knowing which plants to incorporate into the system is still a challenge, and which plants will provide multiple benefits to agricultural producers. *Varronia curassavica* is a medicinal aromatic plant that gathers such characteristics. It's observed to attract a variety of natural enemies including green lacewings (Neuroptera: Chrysopidae). Given the importance of these predators, we explored whether *V. curassavica* provides resources for *Ceraeochrysa cubana*, a Chrysopidae specie commonly found in several Neotropical agroecosystems, including coffee. We also investigated the possibility of *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), a key coffee pest, get benefits by feeding on *V. curassavica* nectar. It is known that larvae of *C. cubana* prey on eggs and pupae of CLM. We conducted microcosm laboratory experiments to determine whether survival and reproduction of *C. cubana* and CLM and were influenced by the presence of *V. curassavica* inflorescences. Survival of *C. cubana* adults was not influenced by *V. curassavica* inflorescences, but predator larvae survive longer when inflorescences were present. Coffee leaf miner survival was not influenced by *V. curassavica*, but its intrinsic growth rate was lower in the presence of the plant inflorescences. Our results provide evidence that *V. curassavica* may enhance the populations of *C. cubana* or help sustain populations in coffee crops. Moreover, the plant negatively affects pest fitness. Therefore, the introduction of *V. curassavica* in coffee crops is a promising strategy to be adopted by coffee farmers aiming to manage CLM populations.

Keywords: Alternative resources, *Varronia curassavica*, Chrysopidae, *Leucoptera coffeella*.

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[1] Chapter formatted for publication in the international peer-reviewed journal, BioControl

1. INTRODUCTION

The strategic introduction of plant species to the main crop can favor the attraction, establishment and effectiveness of pest natural enemies. These plants should provide essential or supplementary resources for beneficial species, including food, shelter and oviposition sites, contributing to pollination and pest control services (Landis et al., 2000; Gurr et al., 2003; Venzon et al., 2006; Tscharntke et al., 2008). However, plants selected for this purpose should: be easily controlled without competing for water and nutrients with the crop, survive in the crop area or around the crop with minimal maintenance, be easily handled and have the ability to compete with non-crop plants, and importantly, plants must provide desired resources to natural enemies of target pests and not host or supply food for pestiferous herbivores (Venzon et al., 2006; Lavandero et al., 2006; Venzon and Sujii, 2009; Souza et al., 2010; Venzon et al., 2011; Chen et al., 2020).

The provision of plant derived food, such as pollen and nectar, can increase the diversity and abundance of natural enemies and reduce herbivory within cropping systems (Tylianakis al., 2004; Koptur, 2005, Letourneau et al., 2011, Rezende et al., 2014). When prey are scarce, non-prey resources can help sustain natural enemies populations in the area until the next increase in pest population, when natural enemies will already be in the area to contribute to control (Landis et al., 2005; Wäckers, 2005; Lundgren et al., 2008; Rezende et al., 2014; Batista et al., 2017). Some natural enemies depend on plant provided food during non-carnivorous life stages (e.g., green lacewings, hover flies). Others, use plant-food to supplement diets of suboptimal prey (Venzon et al., 2006; 2011; Amaral et al., 2013). For example, inga trees grown within coffee crops provide nectar to parasitoids, which increases populations in the area (Rezende et al., 2014). However, some herbivores are also able to feed on pollen and nectar (Van Rijn et al., 2002; Baggen et al. 1999, Rosado et al., 2021). Therefore, understanding the relative value and effect of different plants on herbivores and associated natural enemies is decisive for implementing effective conservation biological control strategies (Venzon et al., 2001).

Varronia curassavica Jacq. (Cordiaceae), known as "erva-baleeira", is a medicinal plant, aromatic perennial shrub, native to Brazil (Gasparino and Barros, 2009). It blooms all year round ensuring a continuous supply of resources for insects, besides survive in the crop area with minimal maintenance. It has terminal inflorescences in spikes with white flowers (Fig 1.) and *V. curassavica* leaves can be sold to industries that manufacture herbal medicines, mainly as anti-inflammatory cream (Magalhães, 2010; Brandão et al., 2015). Many insects are attracted to *V. curassavica*, including: pollinators (Apidae) and predators, mainly Formicidae and Vespidae (Brandão et al., 2015; Hoeltgebaum et al., 2018). During observations in coffee fields where *V. curassavica* was present, we noted the constant and abundant presence of predatory green lacewings (Neuroptera: Chrysopidae) (E.F. Martins, Personal, observation).

Chrysopidae is an important biological control agent of pests in many agricultural systems (Senior and McEwen, 2001; Ecole et al., 2002; Venzon et al., 2006; Pappas et al. 2011; Barbosa et al., 2019). In the coffee production systems of Brazil, *Ceraeochrysa cubana* (Neuroptera: Chrysopidae) (Martins et al., 2019, Ribeiro et al., 2014) is a common species whose larvae feed on a variety of prey (Canard and Principi, 1984, Venzon and Carvalho, 1992). Adults use pollen, and sugary foods, such as nectar and honeydew (Venzon et a., 2006; Venzon and Carvalho, 1992). A recent study showed that *C. cubana* larvae is able to prey on immature stages of coffee leaf miner (CLM) *Leucoptera coffeella* in laboratory, since the egg stage until pupae showing itself as an efficient CLM predator (Chapter 1, Martins et al 2021). Therefore, integrating plants, such as *V. curassavica*, that attract Chrysopidae species may contribute to conservation biological of CLM.

Here we evaluate the potential value of *V. curassavica* for improving conservation biological control of CLM in coffee agroecosystem. We investigated whether *V. curassavica* selectively provides resources for the green lacewing without benefiting CLM. Adults of CLM could feed on the flower nectar, and in laboratory studies a diet of 10% sucrose solution increased the number and viability of the CLM eggs, and females laid 2.1 times more eggs (Nantes and Parra 1978, Parra, 1985). Therefore, to establish *V. curassavica* as a suitable plant for incorporation into the coffee system we must determine effects on natural enemies and on the target pest. Specifically, we determined the survival of *C. cubana* larvae and adult in the presence of *V. curassavica* inflorescences. Additionally, we measured the survival and reproduction of CLM adults in the presence of *V. curassavica* inflorescences.

2. MATERIAL AND METHODS

2.1 Insect rearing

2.1.1 Green lacewing

Ceraeochrysa cubana rearing was maintained under a 12h photoperiod regime, at 23 ± 1ºC and 70 ± 2% RH. The colony was established in the Laboratory of Entomology at Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG). Our laboratory colony of *C. cubana* has been maintained with genetic diversity for 15 years with periodic introduction of wild individuals from the field. We reared the adult lacewings in cylindrical PVC cages (10 cm in diameter and 20 cm in height) and fed them with an artificial diet based on yeast and honey (1:1) offered on a parafilm stripe hung inside the cage (Venzon et al., 2006). We provided water on a piece of cotton soaked and placed inside a 10 mL vial. Food and water were replaced twice a week. We collected eggs of *C. cubana* from the cages by cutting their pedicels and transferred them to glass tubes (2.5 x 8.5 cm). We fed the newly emerged larvae with eggs of *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae) until reaching the pupal phase.

2.1.2 Coffee leaf miner

Leucoptera coffeella rearing was maintained in the same laboratory and under the same climate conditions as described above. The colony was established using material field collected from active mines leaves from plants in an experimental coffee crop located at the Campus of the Federal University of Viçosa (UFV), Viçosa, MG, Brazil. To keep the leaves turgid, we inserted the petioles into foam sections soaked in water and placed them inside 20 x 10 cm plastic containers. We kept the leaves inside transparent acrylic cages (40 x 40 x 40 cm). Daily, we removed newly emerged adults and transferred them to new cages with clean coffee leaves to continue the rearing (Adapted of Reis Jr. et al., 2000).

2.2 Survival and reproduction of C. cubana adults and larvae in the presence of V. curassavica inflorescence

For the experiment with *C. cubana* adults, we collected *C. cubana* pupae from the rearing and placed them individually in microtubes (1.5 mL) until the emergence of adults. We sexed the newly emerged adults under microscope by the shape the of the terminal abdominal segment as described by Martins (2014), to form couples. We placed each *C. cubana* couple inside a plastic pot (500 mL) and offered the following treatments (n=26/treatment): i) inflorescence of *V. curassavica* with open flowers (circa of 5 flowers) and water; ii) only water, as a negative control; and iii) yeast and honey diet (1:1) and water, as a positive control. We inserted the peduncle of *V. curassavica* inflorescence into a small vial of 20 mL with water to maintain turgidity. For the negative control, we offered water through of cotton soaked in water in a 3 mL plastic cap. For the positive control we placed the artificial diet of yeast and honey on a strip of parafilm taped to the wall of the pot and cotton soaked in water in a plastic cap of 3 mL. We covered the arenas with a piece of PVC plastic sheet. Every morning we conducted the evaluations and we replaced the inflorescences for fresh ones, to ensure that the quality and quantity of the resource supplied was consistent (Hoeltgebaum et al, 2018). Thus, daily we assessed the survival of males and females, and the female oviposition, until the death of both or for a maximum of 30 days.

For the assay of *C. cubana* larvae, we used first instar larvae. We placed each *C. cubana* larvae inside a plastic pot (500 mL) and offered to them the following treatments (n=30/treatment): i) inflorescence of *V. curassavica* with open flowers (circa of 5 flowers) and water; ii) only water, as a negative control; and iii) Eggs *E. kuehniella* and water as a positive control. We inserted the peduncle of *V. curassavica* inflorescence into a small vial of 20 mL with water to maintain turgidity. For the negative control we offered water through of cotton soaked in water in a 3 mL plastic cap. For the positive control we offered *E. kuehniella* eggs each three days of the pot and cotton soaked in water in a plastic cap of 3 mL. We covered the arenas with a of PVC plastic. Every morning we conducted the evaluations and we replaced the inflorescences for fresh ones, to ensure that the quality and quantity of the resource supplied was consistent (Hoeltgebaum et al., 2018). Thus, daily we assessed the survival of *C. cubana* larvae until the death or emergence of adults.

As lacewing larvae are generalists it is possible that they feed on pollen and nectar of *V. curassavica,* as well as other soft-bodied insects that inhabit the inflorescences or even honeydew secreted by Hemiptera insects (Pappas et al., 2011; Dhandapani et al., 2016; Venzon et al., 2006). For this reason, at the same time of the collection of *V. curassavica* inflorescences to the experiments of *C. cubana* larvae survival, we also collected other inflorescences to access the arthropods inhabiting *V. curassavica*. We collected the inflorescences in a *V. curassavica* plant located at Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG). Thus, every morning we collected five fresh inflorescences and directly individualized in plastic pots with alcohol 70%. After five minutes, each inflorescence was removed and the arthropods that remained in the alcohol were stored and later identified.

2.3 Survival and reproduction of coffee leaf miner adults in the presence of V. curassavica inflorescences

We used laboratory reared CLM pupae and we transferred them into individual microtubes (1.5 mL) until adult emergence. We sexed newly emerged adults based on the shape of the terminal abdominal segment as described by Notley (1956) and we formed couples. We placed each couple inside plastic pots (500 mL) and offered the following treatments (n=30/treatment): i) one inflorescence of *V. curassavica* with open flowers (circa of 5 flowers) and one coffee leaf and; ii) one coffee leaf, as control. We inserted separately the peduncle of *V. curassavica* inflorescence and the petioles of coffee leaves in small vials containing 20 mL of water to maintain their turgidity. We covered the arenas with PVC plastic. Every morning we conducted the evaluations and we replaced the inflorescences and the coffee leaves for fresh ones, to ensure that the quality and quantity of the resource supplied was consistent (Hoeltgebaum et al, 2018). We assessed the survival of males and females daily until their death. In addition, examined the removed coffee leaves for CLM eggs under microscope. Daily observations of CLM allowed us to determine the pre-oviposition and oviposition period of females and survivorship of both males and females.

2.4 Data analyses

To determine the survival of *C. cubana* we used the presence of *V. curassavica* inflorescence *vs* diet of yeast and honey (positive control) *vs* water (control) as explanatory variable for composing the models. For the survival of *C. cubana* larvae we used the presence of *V. curassavica vs E. kuehniella* eggs (positive control) *vs* water (negative control) as explanatory variable for composing the models. We analyzed the survival of *C. cubana* adults and larvae (variables response) by a censored Weibull distribution and compared by ANOVA χ^2, and later we compared by ANOVA pairwise the survive means between treatments

(Crawley, 2007). Due to the fact that *C. cubana* females oviposited only in the presence of yeast and honey diet (positive control) we did not use this oviposition data in the analyses.

To determine whether CLM population growth is negatively affected by the presence of V. *curassavica*, we used the data on reproduction and longevity of CLM to estimate the intrinsic growth rate (r_m) of the pest on two treatments with inflorescence and without inflorescence of *V. curassavica*. Data on survival and development of juvenile and on sex ratio (0.56) were taken from Jaramillo et al. (2019). We calculated and expressed the r_m of CLM as the number of females per day using the Lotka equation (Carey, 1993):

$$\sum_{x=0}^{T} l_x m_x e^{r_m(x+1)}$$

where *T* is the oldest age class, *lx* is the proportion of surviving females from birth to age *x* and *mx* is the number of female progenies produced per female at the midpoint of the interval *x* to *x+1*.

To determine the survival of CLM adults we used the presence of *V. curassavica vs* water as explanatory variable for composing the models. We analyzed the survival of CLM adults (variable response) by a censored Weibull distribution and compared by ANOVA χ^2 (Crawley, 2007). For all analyses of survival, we used Kaplan-Meier graphs to illustrate the survivorship curves. We analyzed all data in the software R (R Development Core Team, 2018).

3. RESULTS

3.1 Survival of C. cubana adults in the presence of V. curassavica inflorescences

Feeding treatments had a significant effect on survival of *C. cubana* females ($\chi^2 = 207$; DF= 2, $p<0.05$). *Ceraeochrysa cubana* females fed on a diet of yeast and honey survived longer compared to females in the presence of *V. curassavica* inflorescences (z= -10.19; DF=1; p< 0.05; tab. 1; fig 2a) and in the presence of water (z=13.18; DF=2; $p<0.001$; tab. 1; fig. 2a). The survival of *C. cubana* females in the presence of *V. curassavica* inflorescences did not differ significantly from females in the presence of water (z= 1.68; DF=2; p> 0.05; tab. 1; fig. 2a). The survival of *C. cubana* males also differed among treatments (χ^2 =146.75; DF=2, $p<0.05$; tab. 1; fig. 2b). *Ceraeochrysa cubana* males fed on yeast and honey diet showed longer survival in relation the males in the presence of inflorescences of *V. curassavica* (z= -10.95; DF=1; $p<0.05$; tab. 1; fig. 2b) and in the presence of water (z= 13.481; DF=2; $p<0.001$; tab. 1; fig. 2b). The survival of *C. cubana* males in presence of *V. curassavica* inflorescences did not differ significantly from males in the presence of water (z= 0.738; DF=2; p> 0.05; tab. 1; fig. 2b). Neither females nor males survived longer than seven days, except in the diet of yeast plus honey diet. Only *C. cubana* females fed on yeast and honey diet laid eggs preventing further analysis of these data.

3.2 Survival of C. cubana larvae in the presence of V. curassavica inflorescences

Survival of *C. cubana* larvae differed when offered treatments of prey, *V. curassavica* inflorescence or water (χ^2 = 92.23; DF=2; $p<0.05$; tab. 2; fig. 3). *Ceraeochrysa cubana* larvae fed *E. kuehniella* eggs showed higher survival in relation to those in the presence of *V. curassavica* inflorescences (z= -2.58, DF=1; p=0.0098; fig. 3) or water only (z= 4.87; DF=2; $p<0.05$; tab. 2; fig. 3). Furthermore, providing *V. curassavica* inflorescences, as compared to water only, increased the survival of *C. cubana* larvae (z=8.18; DF= 2; p<0,05; tab. 2; fig. 3).

Additionally, larvae completed their life-cycle in the presence of *E. kuehniella* eggs or *V. curassavica* inflorescence (i.e. larvae-pupae-adult; fig.3). We identified the following arthropods inhabiting *V. curassavica* inflorescences: Coleoptera larvae, *Delphastus pusillus* (Coleoptera: Coccinellidae) adults, Lygaeidae (Hemiptera) adults, Aphididae (Hemiptera), nymph and adult of *Orius insidiosus* (Hemiptera: Anthocoridae), Tingidae (Hemiptera), *Brachymyrmex* spp. (Hymenoptera: Formicidae: Formicinae) and thrips *Frankliniella* spp. (Thysanoptera: Thripidae: Thripinae).

3.3 Survival and reproduction of coffee leaf miner adults in the presence of V. curassavica inflorescences

The presence of *V. curassavica* inflorescences did not increase the survival of CLM females or males compared to the control without inflorescences (χ^2 = 3.22, DF= 1, p= 0.07, χ^2 = 0.22, DF= 1, p= 0.64; tab.3; fig. 4 a,b). There was no significant difference in the pre-oviposition period of CLM in the presence or absence of *V. curassavica* inflorescences (z= -0.769; DF= 1; p=0.442; tab. 3). In the presence of *V. curassavica* inflorescences females, overall, laid fewer eggs than in the absence of inflorescences (z= -7.051; DF= 1; $p<0.05$; tab. 3). However, the average number of eggs laid per female per day was not significantly different between treatments (z= -1.379; DF=1; p=0.168; tab. 3). Combining survival and reproduction results, the estimated intrinsic growth rate (r_m) of CLM was lower in the presence of *V. curassavica* inflorescences (r_m= 0.1004) than control (r_m= 0.1207).

4. DISCUSSION

Our combined results suggest that *V. curassavica* is a promising plant for improving conservation biological control of CLM by providing alternative resources to predators, without benefiting the pest. Firstly, integrating different plants into production areas should have net negative effects on pests and not to their natural enemies. In our study, the pest, CLM, had a lower intrinsic growth rate (r_m) in the presence of *V. curassavica* inflorescences than when the inflorescences were absent thus, fewer individuals will be added to the population. Although some Lepidoptera species have been reported to visit inflorescences of *V. curassavica,* there are no records of the effect of such feeding on survival and reproduction of these visitors (Hoeltgebaum et al., 2018; Brandão et al., 2015). *Varronia curassavica* is a heterostylic plant producing two different floral forms in the same plant, with differences between the height of the stigma and the anthers (Hoeltgebaum et al., 2018). In some plant species the nectar glands may not be accessible to insects making them unable to get the nutrients from the flowers (Patt et al., 1997, Vattala et al., 2006; Gardarin et al., 2018; Hatt et al., 2019). Additionally, only flowers whose floral architectures are compatible with a given insect's morphology and floral foraging behavior can provide nutrients to that insect (Patt et al., 1997). Despite the floral architecture, that could be one factor preventing CLM adults from accessing enough nutrients, we believe that the negative effect on CLM growth rate is more related to plant volatiles. A possible explanation would be that *V. curassavica* volatiles hinder the mating of CLM either by releasing compounds in the environment or being present with the nectar of the studied plant. Studies show that the reproductive success of females of various species of Lepidoptera depends on nutrients provided by the male at the time of copulation for egg production (Greenfield, 1982; Rogers and Marti, 1996, Michereff et al., 2004). Therefore, it is possible that males of CLM suffer some inhibitory effect in the presence of *V. curassavica*, impairing the transfer of nutrients at the time of mating and, consequently, the females oviposited less. *Varronia curassavica* is a medicinal and aromatic species that stores essential oils in leaves and other parts of the plant (Ventrella and Marinho, 2008). The volatile compounds in the essential oil of plants can act as mediators for the repellency and attraction of arthropods. One of the major components present in the essential oil of *V. curassavica* is alpha-pinene, which is reported as an insect repellent (Nerio et al., 2010; Martins, 2017;

Andrade et al., 2021). However, in the experiment each CLM couple was kept in closed pots. If *V. curassavica* would induce repellence on CLM, the adults would not feed on *V. curassavica* and consequently the females would oviposit similar as in control. *Varronia curassavica* is a species native to Brazil (Gasparino and Barros, 2009), while CLM is originally from the African continent, therefore exotic (Green, 1984). Thus, both do not have a shared life history, which can disadvantage CLM populations due to the non-adaptation of CLM in assimilating alternative resources of *V. curassavica*. Besides, CLM is monophagous pest insect in the larva stage and this shows its close relationship with coffee, which also originates in Africa (Souza et al., 1998; Pantoja-Gomez et al., 2019).

Survival of *C. cubana* adults was not influenced by the presence of *V. curassavica* inflorescences. Also, females of the predator did not lay eggs in the presence of *V. curassavica* inflorescences. One possible explanation is that females need a higher nutrient concentration of protein and carbohydrates than present in the pollen and nectar of *V. curassavica* flowers to oviposit (Venzon et al., 2006). However, the absence of positive effect on adult longevity also suggest that adults did not successfully feed on this plant provided food, as this species is known to survive for longer periods feeding only on sugary foods (Venzon and Carvalho1992). Thus, the non-increase in *C. cubana* adult survival in the presence of *V. curassavica* could be explained by the fact that either nectar or pollen, or both, are not accessible for adults due to the morphology of the flowers and of *C. cubana* mouthparts. In studies with parasitoids and generalist predators, the access to nectar was predominantly influenced by flower morphology, nectar quality and by the mouthpart structure of insects (Baker and Baker, 1983; Jervis, 1998; Baggen et al., 1999; Patt et al., 1997; Wäckers, 2004; Hatt et al., 2019).

The presence of *V. curassavica* inflorescences increased the survival of *C. cubana* larvae. It seems as the larvae of *C. cubana* successfully feed on pollen and nectar of *V. curassavica*. Chrysopidae larva species feed on by piercing their prey with their long, modified mandibles and sucking the juices out of them (Canard and Volkovich, 2007). Likewise, the survival of *C. cubana* increased in the presence of basil flowers (Batista et al., 2017). Chrysopidae species are reported also to feed on aphids, thrips and other soft-bodies insects (Albuquerque et al., 2001; Venzon et al., 2006; Sarkar et al., 2019). These insects were found in the inflorescences, but at low densities. Therefore, we believe that *C. cubana* larvae preyed on Coleoptera larvae, aphids and thrips inhabiting the *V. curassavica* inflorescences, but that they acquired part of their nutrients from pollen and nectar. The 30 larvae of *C. cubana* in contact with the inflorescences of *V. curassavica*, 17 pupated up and 13 became healthy adults confirming that the presence of this medicinal species complements the diet of *C. cubana*. In this sense, *V. curassavica* offers different resources improving the survival and development of *C. cubana* larvae. Likewise, larvae of *C. cubana* survived longer in the presence of basil flowers than in the absence (Batista et al., 2017).

Chrysopidae are known by its generalist feeding habits (Canard and Principi, 1984, Venzon and Carvalho, 1992; Albuquerque, 2009; Pappas et al., 2011). They can exploit different plant species for food and used them during the adult stage or during larvae, to complement their diet. Both, *C. cubana* and *V. curassavica* are native from Brazil, that is both have a shared life history, therefore *C. cubana* have more probability in assimilating alternative resources of *V. curassavica*. Although in our experiment we have not found effect of *V. curasssavia* on adults, a possible explanation for the plant attractiveness of Chrysopidae species in the field to could be related to the constitutive volatiles released by the plants, even when they are not flowering. Batista et al. (2017) reported that females of *C. cubana* are attracted to basil plants even in the absence of flowers, suggesting a possible role of volatile organic compounds. Other alternatives for the attractiveness of adults of *C. cubana* in the field by *V. curassavica* would be the provision of honeydew from hemipteran, of shelter or of oviposition sites and food for their offspring, as larvae benefit for the plant (Togni et al., 2016, Zhu et al., 2005).

The introduction of plants in an agricultural system should be carried out from an ecological perspective, with knowledge of the multiple interactions between the components of the system to ensure long-term agricultural sustainability (Venzon et al., 2019). Our study demonstrates in how a plant could be evaluated for conservation biological control purpose. In view of these perspectives, our results support that *V. curassavica* provides selective resources for a natural enemy (*C. cubana)*, without benefiting the pest (CLM). This medicinal plant provides non-prey and prey to natural enemies which can be used when prey populations in the main crop are scarce. Our findings suggest that associating *V. curassavica* with coffee crops is a promising strategy to be adopted by coffee farmers for the CLM management.

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Tables

Table 1. Survival of *Ceraeochrysa cubana* females and males in the presence of *V. curassavica* inflorescence, artificial diet or eggs or water.

	Treatments			
Parameter	***V. curassavica* inflorescence**	**Artificial diet**[1]	**Water**[2]	**p-value**
Survival of *C. cubana* females	1.38 ± 0.40 **b**	27.42 ± 0.37 **a**	1.23 ± 0.09 **b**	< 0.05*
Survival of *C. cubana* males	1.57 ± 0.31 **b**	23.19 ± 0.26 **a**	1.46 ± 0.12 **b**	< 0.05*

Means (± SE) of parameters for each treatment compared by χ^2 test ($p<0.05$)

Means followed by equal letters in lines do not differ from each other

[1] Positive control to *C. cubana* adults (Yeast and honey)

[1] Negative control to *C. cubana* adults

Table 2. Survival of *Ceraeochrysa cubana* larvae in the presence of *V. curassavica* inflorescence or *E. kuheniella* eggs or water.

	Treatments			
Parameter	***V. curassavica* inflorescence**	***E. kuheniella* eggs**[1]	**Water**[2]	**p-value**
Survival of *C. cubana* larvae	13.6 ± 1.84 **b**	16.36 ± 1.85 **a**	3.86 ± 0.13 **c**	< 0.05*

Means (± SE) of parameters for each treatment compared by χ^2 test ($p<0.05$)

Means followed by equal letters in lines do not differ from each other

[1] Positive control to *C. cubana* larvae

[2] Negative control to *C. cubana* larvae

Table 3. Coffee leaf miner reproduction in the presence and in the absence of *V. curassavica* inflorescence.

Parameter	**Treatment** ***V. curassavica*** **inflorescence + Coffee leaf**	**Coffee leaf**	**p-value**
Pre-oviposition period (days)	2.16 ± 0.49	2.70 ± 0.40	> 0.05^{ns}
Total number of eggs per female	17.56 ± 3.86	26.13 ± 4.58	< 0.05*
Number of eggs per females per day	2.20 ± 0.47	3.48 ± 0.60	> 0.05^{ns}
Survival of coffee leaf miner female	8.30± 0.11	6.97± 0.08	> 0.05^{ns}
Survival of coffee leaf miner male	7.30±0.12	6.23±0.09	> 0.05^{ns}

Means (± SE) of parameters for each treatment compared by z test ($p<0.05$)

Figures

Figure 1. *Varronia curassavica* inflorescence (Photo: Elem Fialho Martins).

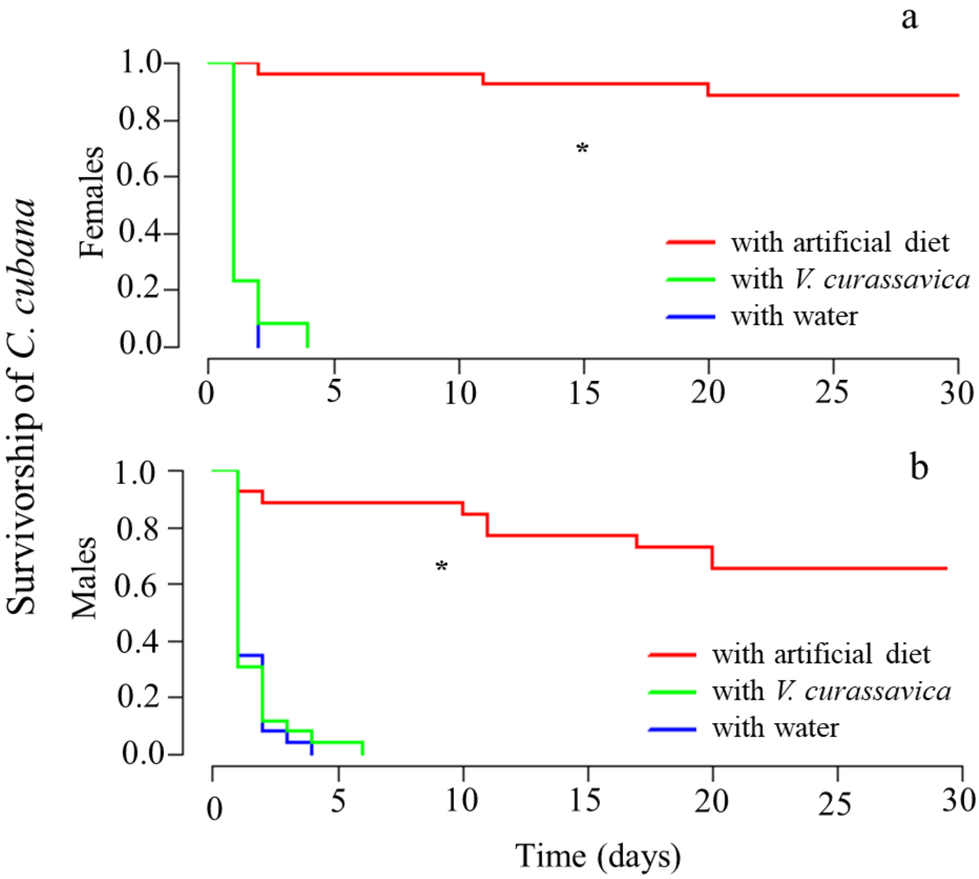

Figure 2. Survivorship of *C. cubana* (a) females (χ^2= 207; DF= 2, p<0.05) and (b) males (χ^2 =146.75; DF=2, p<0.05) in the presence of *V. curassavica* inflorescence, only water and in the presence of artificial diet of yeast and honey.

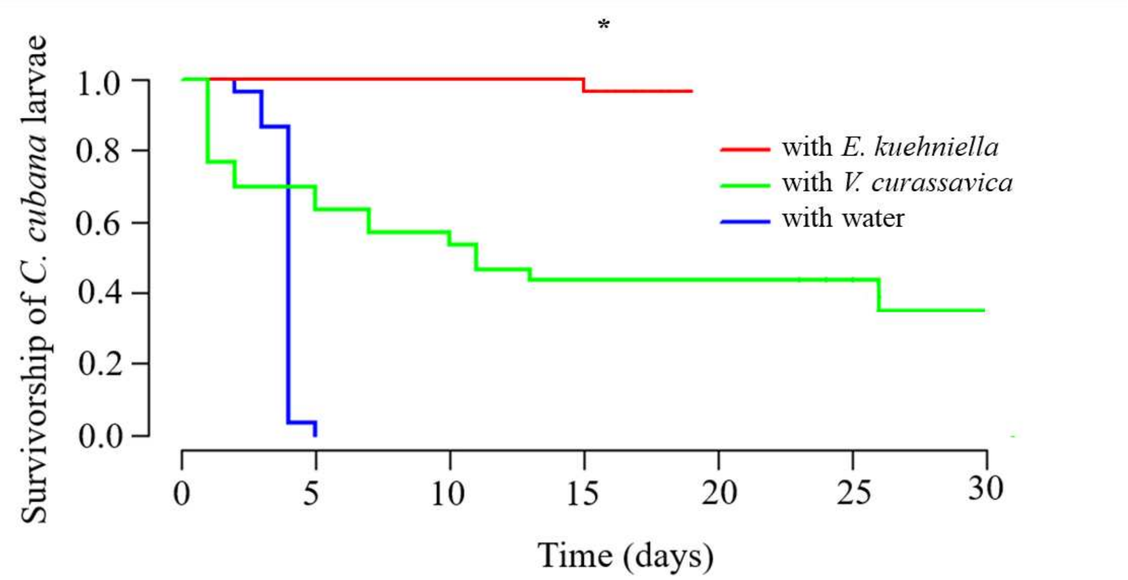

Figure 3. Survivorship of *C. cubana* larvae in the presence of *V. curassavica* inflorescence, only water and in the presence of *E. kuehniella* eggs ($\chi^2 = 92.23$; DF=2; $p<0.05$).

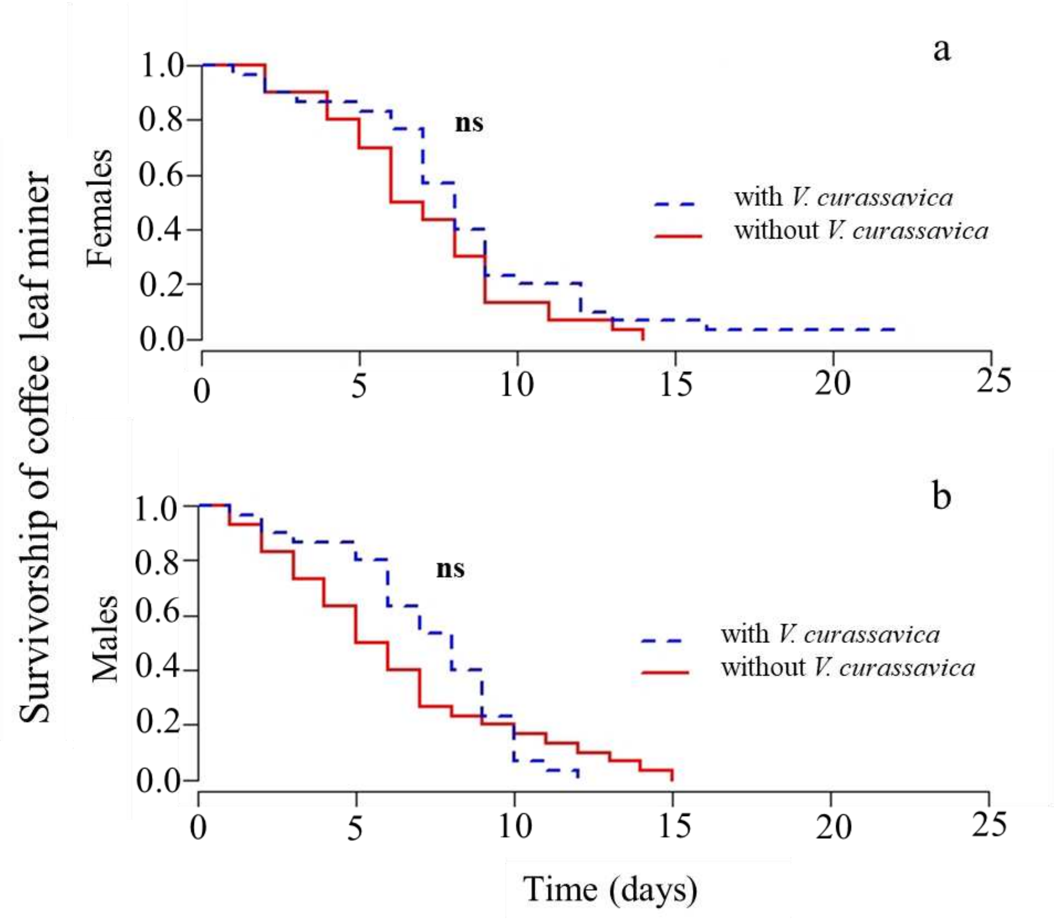

Figure 4. Survivorship of coffee leaf miner (a) females (χ^2 = 3.22, DF= 1, p= 0.07) and (b) males (χ^2= 0.22, DF= 1, p= 0.64) in the presence and in the absence of *V. curassavica* inflorescence.

Chapter III[1]

Selective habitat diversification enhances biological control of coffee leaf miner in a coffee agroecosystem

ABSTRACT

Landscape simplification blended with the indiscriminate use of pesticides often reduces the efficiency of natural biological pest control. “Re-wilding” production landscapes through the introduction of selected plants (strategic habitat diversification) and maintenance of non-crop areas has the potential to restore biodiversity mediated ecosystem services (i.e. pollination, biological control). For example, perennial monoculture systems, such as coffee, provide permeant habitat where addition of attractive perennial flowering plants should enhance biological control. Here, we propose and examine a diversified coffee system combining the introduction of selected perennial plants (*Inga edulis*, *Senna macranthera* and *Varronia curassavica*) with encouraged growth of non-crop plants. We evaluated whether a diversified system increases the abundance and effectiveness of predators and parasitoids and decreases damage of the coffee leaf miner (CLM) *Leucoptera coffeella*. The diversified system had similar abundance and richness of natural enemies as compared to the conventional system lacking habitat enhancements. However, in the diversified system, we observed higher parasitism rates on CLM, and lower infestation of CLM. Finally, our results suggest that strategic diversification increases biological control efficacy of CLM in coffee crops.

Keywords: Conservation biological control; *Inga edulis*, *Senna macranthera*; *Varronia curassavica*; Natural enemies, *Leucoptera coffeella*.

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[1] Chapter formatted for publication in the international peer-reviewed, Journal Agriculture, Ecosystems and Environment.

1.INTRODUCTION

Coffee (*Coffee* spp.) is a native plant to the understory of the highland forests of Ethiopia and Sudan and it was originally cultivated close to native forest trees, fruit species, and nitrogen-fixing species (Moguel and Toledo, 1999; Anthony et al., 2002; Bandeira et al., 2005). In the mid-1980s and early 1990s, there was an incentive by governments to intensify coffee crops to maximize productivity, which lead to transformation of coffee agroecosystems into coffee monocultures (Rice, 1999). This scenario extends to the present day in several regions of Brazil, que world coffee producer, where large areas are used for coffee monocultures, as in the Cerrado, a Savanna like vegetation and one of the Brazilian Biomes, in the state of Minas Gerais. One the consequences of coffee monocultures is the landscape simplification. Compounded with reduced native vegetation is overuse of pesticides in agriculture, which is linked to reduction in the abundance and diversity of natural enemies and consequently, important ecosystem service, such as pollination and biological control (Krishna et al., 2003; Perfecto et al., 2014, Rusch et al., 2016; Janssen and Van Rijn, 2021). Furthermore, some pests evolve resistance to pesticides (Fragoso et al., 2003; Pereira et al., 2007; Guedes et al., 2016, 2017; Leite et al., 2020, 2021). This is the reality for the coffee leaf miner (CLM) *Leucoptera coffeela* (Guérin-Mèneville, 1842) (Lepidoptera: Lyonetiidae) a key coffee pest in Brazil (Fragoso et al., 2003; Reis et al., 2002). The attack of CLM on coffee causes early senescence of leaves, which causes high losses in yield, weight and quality of coffee, and on the longevity of the coffee plants (Pereira et al., 2007).

Natural enemy species commonly found preying on or parasitizing CLM in coffee crops include: green lacewings, wasps, ants, hymenopteran parasitoids (Ecole et al., 2002; Pereira et al., 2007; De la Mora et al., 2008; Fernandes et al., 2009; Lomelí Flores et al., 2009, 2010; Rezende et al., 2014; Androcioli et al., 2018; Martins et al., 2021). Green lacewings and ants are generalist predators reported to prey on eggs, pupa and pre-pupa of CLM (Ecole et al., 2002;

Martins et al., 2021, De la Mora, 2008; Lomelí Flores et al., 2009). Predatory wasps are known to prey on CLM larvae by tearing mines and removing the larva (Pereira et al., 2007; Fernandes et al., 2009; Androcioli et al., 2018). Parasitoids lay their eggs inside the CLM larvae or pupae (Pereira et al., 2007, Lomelí Flores et al., 2009). Although there are several natural enemy species reported in coffee crops and known to prey on CLM, their populations are often not sufficient to suppress CLM below economic thresholds (Rosado et al., 2021). One of the reasons is the lack of non-agricultural habitats providing alternative prey and non-prey resources (Tscharntke et al., 2012, 2016; Venzon et al., 2006; Olson et al., 2005).

Habitat management is promoted as a solution to enhance resources for natural enemies, and promote ecosystem services such as biological control. Habitat management requires knowledge of plants that provision alternative food and refuge for natural enemies. However, not all plants are suitable for this purpose (e.g. Venzon, 2021). High quality plants should selectively provide accessible resources to natural enemies without benefiting pests (Venzon et al., 2006; Lavandero et al., 2006; Venzon and Sujii, 2009; Chen et al., 2020). Plants selected for habitat management programs should be easily grown, and not compete for water and nutrients with crops (Souza et al., 2010; Venzon et al., 2011; Rezende et al., 2021). Of particular interest for habitat management that benefit arthropods are plants that produce extrafloral nectar and those with long bloom periods providing continuous resources to natural enemies. Extrafloral nectaries are nectar-secreting glands located outside the flowers commonly found tropical plants (Koptur, 2005; Souza et al., 2010; Rezende et al., 2014, 2021). Pollen and nectar from flowers are used by natural enemies to supplement or complement their diet (Olson et al., 2005; Venzon et al., 2006, 2019).

Here, we selected three species of plants to be introduced into coffee crops in the Cerrado, based on their selective provision of food to natural enemies and their compatibility to the coffee crop management of Cerrado: *Inga edulis* Martius (Fabaceae) (Fig .1), *Senna macranthera* DC.

ex Collad. H.S.Irwin and Barneby (Fabaceae) (Fig. 2) and *Varronia curassavica* Jacq. (Cordiaceae) (Fig. 3). The genus *Inga* sp. possess extrafloral nectaries and are known to improve the richness and abundance of natural enemies of CLM (Rezende et al., 2014). Plants of the genus *Senna* sp. are commonly found associated to coffee in agroforestry systems in Zona da Mata-MG, and also present extrafloral nectaries which attract ants (Marazzi et al., 2006, Souza et al., 2010; Marazzi et al.,2013). Both also provide pollen and floral nectar to natural enemies and pollinator during blooming (Falcão and Clement, 2000; Pinheiro et al., 2018). *Varronia curassavica* is a perennial shrub, native of Brazil, and blooms during all year, providing pollen and nectar constantly to natural enemies (Brandão et al., 2015; Martins, 2017; Hoeltgebaum et al., 2018). Additionally, Martins (2017) shows that bees, known by pollinate coffee crops, and different ants and wasps visit the inflorescences of *V. curassavica,* in different hours of the day. Moreover, the author observed the several wasps species feed on *V. curassavica* mature fruits and visiting inflorescences. Besides the perennial plant, we also kept the non-crop plants between the coffee rows. The maintenance of non-crop plants provides food and refuge to natural enemies (Amaral et al., 2013; Venzon et al., 2019).

Our aim is to evaluate the insect community visiting the associated plants with *I. edulis*, *S. macranthera* and *V. curassavica* and their effect on CLM population. To assess the benefits of habitat management for promoting biological control in coffee systems, we compared an experimental diversified system to common conventional practices lacking habitat enhancements. For the diversified system, we selected three species of plants to be introduced into coffee crops, based on their selective provision of food to natural enemies and their compatibility with coffee crop management: *Inga edulis* Martius (Fabaceae) (Fig .1a), *Senna macranthera* DC. ex Collad. H.S.Irwin and Barneby (Fabaceae) (Fig. 1b) and *Varronia curassavica* Jacq. (Cordiaceae) (Fig. 1c). (Marazzi et al., 2006, Souza et al., 2010; Marazzi et al.,2013). (Falcão and Clement, 2000; Pinheiro et al., 2018). (Brandão et al., 2015; Martins, 2017; Hoeltgebaum et al., 2018). In addition, vegetation between rows was allowed to grow, forming a diversified system of selected introduced plants and common vegetation observed in coffee. The conventional system lacked the addition of selected plants or between row vegetation and was managed with insecticides. We evaluated whether the diversified coffee system would: (i) increase the abundance and richness of Chrysopidae species and social wasps, known as predators of CLM; (ii) improve the parasitism rate of CLM; and (iii) decrease the infestation rate of CLM.

2. MATERIAL AND METHODS

2.1 Field experiment

We conducted the experiment during the years 2019, 2020 and 2021, at the Experimental Farm of the Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG), in the municipality of Patrocínio, state of Minas Gerais, Brazil (18 ° 59’48” S, 46 ° 59’ 00” O), located in the Cerrado Biome. This region presents the precipitation around of 1600 mm in average annual (Inmet, 2021).

In an established coffee field, we established two treatments: (1) a diversified coffee system; and (2) a conventional coffee system (monoculture), both with *Coffea arabica*. We arranged the treatments in three blocks based on coffee variety and age of stand. In two blocks, the variety of the coffee plants was Catuaí Vermelho IAC 99 and in other block, the variety was Acaiá IAC 474 - 19. The blocks of the Catuaí variety were cultivated since 1993 and the block of the Acaiá since 1987, with “truking” (a practice used to cut coffee plants at about 30 to 40 cm from the ground) in 1998. Both cultivars have the same susceptibility to the most common coffee pests and diseases (Fazuoli et al., 2007). In the two treatment systems (diversified or conventional), coffee was spaced 4 m between lines and 0.5 m between plants. In each block, plots measuring 1080 m^2 were assigned to either diversified or conventional. Within the block, plots were separated by at least 200 m. The minimum distance between blocks was 500 m (Fig. 4).

For the diversified plots, we planted four *I. edulis*, two *S. macranthera* and 12 *V. curassavica* plants, per plot. The seedlings of *I. edulis* and *S. macranthera* were provided by Espaço Botânico at Uberlândia-MG, Brazil, while the *V. curassavica* seedlings were produced from seeds harvested at Experimental Research Station of Epamig, in Oratórios-MG, Brazil. In December 2018, we transplanted the seedlings when they reached 80 cm height. They were placed in two rows of the plot, at each border, with a spacing of 5 m between plants (Fig. 5). No pesticides were sprayed in the diversified coffee systems, except for coffee rust infestation (Copper hydroxide at 1.5 Kg/ha; Appendix 2). We maintained the non-crop plants between coffee rows at a height of 50 cm by rotary cutter mower. Consistent with past research, common non-crop plants found between rows of coffee were: *Gnaphalium spicatum, Conyza bonariensis, Solanum Americanum*, *Ageratum conyzoides, Bidens pilosa* and *Sida cordifolia* (Franzin, 2021). Mineral fertilizers were applied consistent with standard conventional coffee management of the Cerrado (Appendix 1, 2 and 3).

In the plots with conventional systems, pesticides were applied to control CLM infestations using Abamectin (Avermectin) and Thiamethoxam (Neonicotinoid) in December 2018, February 2019, February 2020, January and April 2021 (Appendix 2). Control of coffee rust and non-crop plants followed standard management of conventional coffee (Appendix 3). Mineral fertilizers were applied consistent with standard conventional coffee management of the Cerrado (Appendix 1, 2 and 3).

2.2 Visiting insects of associated plants

To access the insect community attracted by selective plant introductions, we collected the visitors on the extrafloral nectaries of four *I. edulis* and two *S. macranthera,* and on *V. curassavica* inflorescences of four plants per plot. We carried out active collections of the insects, with suction samplers, during five minutes observations each one hour. Every five minutes, three people, one in each block simultaneously, observed a group of either five extrafloral nectaries of *I. edulis,* or three extrafloral nectaries of *S. macranthera* or a branch of *V. curassavica* with open inflorescence, all randomly selected on each plant. After 5 minutes of observation, each person evaluating selected other nectaries or inflorescences on another plant. We carry out collections at three different times of the day (8 a.m., 12 a.m. and 4 p.m.). In total, we sampled in seven collection dates between October 2019 and May 2021. We stored all visiting insects in 70% alcohol for later identification at the family/species level.

2.3 Abundance and richness of green lacewings

To evaluate whether the diversification of coffee crops improve the abundance and richness of Chrysopidae, we sampled coffee plants with an entomological sweep net. Sampling was dome by sweeping the coffee plants along two transects of 30 m, at three different times of the day (8 a.m.; 12 a.m. and 4 p.m.). The transects were in the center of each plot, both in the diversified and in conventional treatments. At the end of each sweeping in each transect, we transferred all captured insects to plastic bags and then take them to the laboratory for sorting. After, the green lacewings were selected from all collected insects, and were individualized, counted and stored in in 70% ethanol. We then send the specimens to a Chrysopidae taxonomist to identify at the species level. In total, we did six collections of Chrysopidae between August 2020 and May 2021.

2.4 Abundance and richness of wasps

To evaluate whether the diversification of coffee crops improve the abundance and richness of social wasps we realized collections in both coffee systems with baited traps. The baited trap consisted of 2 L PET (polyethylene) bottles with four circular holes of 4 cm of diameter, hung on coffee branches of approximately 1.5 m above the ground. We filled the trap with 300 ml of industrialized orange juice and 0.4 ml of propylene glycol to conserve the insects (Medeiros et al., 2019). We placed three baited traps along of one linear transect of 30 m, in the middle the plot. The traps were spaced each 3 m. The traps remained in all coffee plots for 7 consecutive days, in the months of October and December of 2020 and January, March, May and April of 2021. We stored all the captured wasps in 70% ethanol for further send the specimens to identification at the species level.

2.5 Parasitism rate of coffee leaf miner

To evaluate whether the diversified coffee system improve the parasitism rate of CLM, we collected mined leaves from coffee plants in both systems. We collected four mined leaves in each 24 plants per plot, in three plants per line, in eight lines. To ensure that the leaf miners had not been attacked by predatory wasps and that parasitoids had not emerged yet, we chose leaves with intact mines. We collected in different distances (4, 8, 12 and 16 m) from *I. edulis*, *V. curassavica* and *S. macranthera.* We followed the same collection pattern in the conventional system, with collection in 24 plants in three plants per row and in eight rows of coffee. In total, there were six collection date, in March 2019, June, September and November 2020, and June and August 2021. After each collection, we carried the coffee leaves to the laboratory to individualized them. We incubated each mined leaf in a separate plastic pot (500 mL) covered to PVC film in the laboratory and we kept them until the emergence of leaf miners or parasitoids. To maintain the coffee leaves turgidity, we used a small container of 5 mL covered with parafilm and after made a hole with tweezers, we put water with a dropper and inserted the petioles of the coffee leaves in the hole in contact with water (Pereira et al., 2007). Pots were examined until 30 days from individualization. We recorded the number of CLM adults and parasitoids emerged. After, we stored the parasitoids in 70% ethanol and we identified at the species or genus level. We calculated the parasitism rate of CLM using the following formula:

$$\text{Parasitism rate (\%)} = \frac{\text{Total number of emerged parasitoids x 100}}{\text{(Total number of parasitoids + CLM)}}$$

2.6 Coffee leaf miner infestation rate

In order to compare the infestation by CLM in diversified and conventional coffee systems we evaluated its damage by collecting 192 coffee leaves per plot, in six plants per line, in eight lines, at each sampling date. We collected the coffee leaves in different distances (4, 8, 12 and 16 m) from *I. edulis*, *V. curassavica* and *S. macranthera*, but in the same time was randomly, because the each collect date, we sampling coffee leaves in different plants. We collected four distal leaves, from the third pair of leaves, from two different branches per coffee plant (adapted from Souza et al., 1998). We sampled two branches in the east and two in the west position due to due to the high-density coffee planting which refrains us to collect in the north and south directions. In the conventional plots, the collection followed the same pattern as in the diversified areas. Thus, in each sampling date we collected 1152 coffee leaves. We kept the coffee leaves in paper bags and later examined in the laboratory to assess the active mines (infestation) and inactive mines (preyed mines, parasitized mines or emergence of CLM adults). By examining the active mines, we assessed the real infestation of CLM. We calculated CLM infestation rate using the following formula:

$$\text{CLM Infestation rate (\%)} = \frac{\text{Number of coffee leaf with active mines x 100}}{\text{Total number of collected coffee leaves}}$$

By observing the inactive mines under a microscope, we assessed preyed mines and the healthy mine, being the later those whose CLM larvae left the mine to pupate on the plant. The attack on mines by predatory wasps is easily distinguishable, due to the torn mine surfaces and absence of CLM larvae or by the presence of remaining larvae with signs of predation (Pereira et al., 2007). The exit holes of CLM larvae are characterized by a "half-moon" shape. We calculated the proportion of coffee leaves with inactive mines per plant using the same formula describe above, but replacing active for inactive mines. After we calculated the proportion of preyed mines from the total number of inactive mines.

Additionally, from the collected leaves, we evaluated coffee rust infestation. The coffee rust *Hemileia vastatrix* Berk and Broome (Basidiomycota, Pucciniales) is a fungal disease that also causes coffee leaf fall. These falls directly affect coffee productivity. Therefore, it is extremely necessary also to evaluate the proportion of rust, because productive decreases under severe rust attack (Zambolim, 2016). Coffee rust infestation was evaluated by number of coffee leaf with presence of small patches of pale-yellow (Rayner 1961; Nutman and Roberts 1963; Montoya and Chaves 1974). The infestation by rust was calculated similar to infestation by CLM, however with the number of leaves with presence of rust. In total, we did 13 samplings of coffee leaves to all analyses from item 2.6, between January 2019 and August 2021.

2.7 Statistical analysis

We analyzed the data separately for each of the response variables. Because we sampled the coffee plants within the same blocks over time, we used block as a random factor. For all analysis we used a model simplification process by 'AICcmodavg' package (Mazerolle and Linden, 2019) and we determined the minimum adequate model(s) by comparing Akaike Information Criterion corrected (AICc) values to all the analysis.

To analyze the effect of coffee system in the abundance and richness of predatory green lacewings, commonly found in the plots during the in diversified and conventional coffee systems we used Generalized linear mixed model (GLMM) with a negative binomial error distribution. We defined the systems (diversified and conventional) as a fixed effect and the block as a random effect. We calculated the variation in abundance and richness of predatory wasps by GLMM with Poisson distribution and we defined the systems (diversified and conventional) and the collecting times as fixed effects and the block as a random effect. For both analysis we compared the GLMM against null models to attest possible random patterns in the predictor variables. We compared the abundance and richness means by χ^2 test of Analysis of Variance (ANOVA).

We analyzed the effect of coffee systems in the parasitism rate, the CLM infestation, preyed mines, and rust infestation using GLMM with binomial error distribution. To parasitism rate we defined the coffee systems (diversified and conventional) and sampling dates (March 2019, June, September and November 2020, and June and August 2021) as fixed effects, and blocks as random effects. To the CLM infestation, preyed mines, and rust we defined the coffee systems (diversified and conventional) and the years (2019, 2020 and 2021) as fixed effects, and blocks and sampling dates as random effects. We compared the means by χ^2 test of Analysis of Variance (ANOVA). All analyzes were performed in R version 3.6.3 (R Core Team 2020).

3. RESULTS

3.1 Visiting insects in the associated plants

In total, we collected 479 insects on *V. curassavica* inflorescences and on extrafloral nectaries of *I. edulis* and *S. macranthera* (Tab. 1). The most visited plant was *V. curassavica*, with 229 insects, followed by *I. edulis* and *S. macranthera* with 182 and 68 visitors, respectively. Ants were the most abundant insects, totalizing 312 individuals and they were more abundant on *I. edulis* (166 individuals). Most abundant ants were *Brachymyrmex* sp.1 (n=124), *Linepithema* sp.1 (n=61) and *Dorymyermex* sp.1 (n=60). Other insects with importance for coffee were also found but with low abundance, such as Hymenoptera parasitoids as Braconidae (n=21), and *Apis mellifera* (n=16).

3.2 Abundance and richness of green lacewings

In total, we collected 133 green lacewings in both systems, 76 in the diversified coffee and 57 in the conventional (Tab. 2). The most abundant species were *Ceraeochrysa cincta* (Schneider, 1851), followed by *Chrysopodes divisus* (Walker, 1853). There was no significant difference in the abundance (χ^2=2.1996; DF=1; p=0.13805; fig. 6) and richness (χ^2=1.4921; DF=1; p=0.2219; fig. 7) of green lacewings between the diversified and the conventional coffee systems.

3.3 Abundance and richness of wasps

In total, we collected 27 wasps in both systems, 17 in the diversified coffee system and 10 in the conventional coffee system (Tab. 3). The most abundant were *Agelaia pallipes* (n=6) and *Polybia sericea* (n=4) in diversified coffee systems, and *Polybia platycephala* (n=4) and *Agelaia multipicta* (n=3) in conventional coffee systems. There was no significant difference in the abundance (χ^2=1.7728; DF=1; p=0.183033; fig. 8) and richness (χ^2=1.5943; DF=1; p=0.2067; fig. 9) of wasps between the diversified and conventional coffee systems.

3.4 Parasitism rate of coffee leaf miner

From the 3456 collected coffee leaves with intact mines, 389 parasitoids of CLM emerged, 226 in diversified coffee system and 163 in conventional coffee systems (Tab. 4). The most abundant species were *Proacrias coffeae* (Ihering, 1914), 105 individuals in diversified and 108 in conventional coffee systems, followed by *Orgilus niger* (Penteado-Dias), 1999 (n=44), and *Stiropius reticulatus* Penteado-Dias, 1999 (n=35), both in diversified coffee systems. Additionally, 33 parasitoid specimens in diversified coffee system and 15 in the conventional were not identified due to the loss of body parts that prevent identification. The parasitism rate was influenced by the coffee systems (χ^2=21.590; DF=1; p= 0.05) and sampling dates (χ^2=21.590; DF=5; p= 0.05) with highest average in June 2020. The parasitism rate of CLM was higher in diversified coffee systems than in the conventional (χ^2=21.590; DF=1; p= 0.00000338; fig. 10). The percentage of parasitism was 8.27% in diversified coffee system and 6.04% in the conventional.

3.5 Coffee leaf miner infestation rate, preyed mines and rust

In total, we sampled 14, 976 coffee leaves in 2019, 2020 and 2021, in both coffee systems. Due to the existence of interaction between systems (treatments) and year (χ=11.6632; DF= 1; p=0.0029), we analyzed the CLM infestation rate between systems in each year separately. There was no significant difference in the infestation rate of CLM between the diversified and coffee systems in 2019 (χ^2 =2.7199; DF= 1; p=0.09911; tab. 5; fig. 11). In 2020, there was significant difference in the infestation by CLM between systems and the infestation was higher in diversified coffee system (χ^2 = 8.9219; DF= 1; p=0.002818; tab. 5; fig. 11). But in 2021, there was again no significant difference in the infestation by CLM between the diversified and conventional coffee systems (χ^2 =1.2658; DF= 1; p=0.2606; tab. 5; fig. 11). Due to the existence of interaction between systems (treatments) and year (χ=62.664; DF= 1; $p<0.05$), we analyzed the proportion of inactive mines between systems in each year separately. There was no significant difference in the proportion of inactive mines between the diversified and conventional coffee systems in 2019 (χ^2 =51.999; DF= 1; $p<0.05$; tab. 5) and 2020 (χ^2 = 1.1838; DF= 1; p=0.2766; tab. 5). However, there was significant difference in the proportion of inactive mines between systems in 2021 (χ^2 =15.030; DF= 1; p=0.0001058; tab. 5) and it was higher in the diversified coffee system. The preyed mines rate was not influenced by the coffee systems (χ^2=0.0801; DF=1; p=0.777), but was influenced by years (χ^2=15.6264; DF=5; p=0.0004). The rate of preyed mines obtained in 2019 was higher compared to 2020 (t=3.161; DF=5; p=0.0045) and 2021 (t=2.911; DF=5; p=0.0101). However, there was no significant difference between the years of 2020 and 2021 (t=0.070; DF=5; p= 0.9973). Predation accounted for 0.7 % in the diversified coffee systems and 0.8 % in the conventional coffee systems. However, there was no difference in the proportion of preyed mines between systems (χ^2 =0.0801; DF= 1; p=0.777; table. 5).

To rust, due to the existence of interaction between systems (treatments) and year (χ^2 =105.0173; DF= 1; $p<0.05$), we analyzed the rust infestation rate between systems in each year. There was significant difference in the proportion of coffee leaves with rust between the diversified and conventional coffee systems in 2019 (χ^2 = 59.874; DF= 1; $p<0.05$) and 2020 (χ^2 =39.751; DF= 1; $p<0.05$) and it was higher in the diversified coffee system. In 2021, there was no significant difference in this proportion between systems (χ^2 =25.105; DF= 1; $p<0.05$), but it shows a trend to be was higher in conventional coffee system (tab.5).

4. Discussion

Our combined results suggest that the strategic diversification studied here contributes to for a most sustainable production coffee and without economic damage. The population of CLM was kept under the threshold level during the experimental period on both systems. However, in the conventional monoculture pesticides were applied for CLM control and in the diversification not. We found that parasitoids were the main agents that contributed to CLM low level, besides their low densities. Moreover *I. edulis*, *S. macrantera* and *V. curassavica* attracted other important natural enemies of CLM, such as ants. The ants were the most abundant insects found visiting the extrafloral nectaries of *I. edulis*, *S. macranthera* and *V. curassavica* inflorescence. The ants *Solenopsis*, *Pseudomyrmex* and *Camponotus* collected on diversified coffee systems are known as predators of different stages of CLM (Lomelí-Flores et al., 2009). Recently, Botti (2021) reported for the same systems, that the abundance and richness of ants were higher in diversified coffee plots compared to monoculture conventional ones.

Chrysopidae species are commonly found in coffee crops being reported as predator of CLM (Ecole et al., 2002; Ribeiro et al., 2014; Martins et al., 2019, Martins et al., 2021). Despite the absence of green lacewings visiting inflorescences and nectaries in the associated plants, it was one of the insects most observed in the plots during of the field evaluations. The abundance and richness of green lacewings in diversified coffee systems were similar to the conventional coffee systems. These results are opposing our expectations, since we would expect that by adding resources in a specific area, such as pollen and nectar, the predator populations will increase Amaral et al., 2013; Venzon et al., 2019). Additional, green house studies showed that green lacewings survived longer in the presence of extrafloral nectaries of inga seedlings (Rezende, 2014). Also, survival of larvae in laboratory increased in the presence of *V. curassavica* inflorescence (Chapter 2). It is possible that the density of plants was not sufficient to increase the abundance of green lacewings. Besides, the low richness and abundance of some green lacewing species in both coffee systems may be due to the sampling method and to the period of the samplings, because some species have crepuscular and nocturnal habits (Oswald and Machado, 2018). Besides, the occasional presence of non-crop plants in the conventional systems might have influenced the abundance and richness of green lacewings similar the diversified coffee systems (Días, 2014).

Our results showed that the diversified coffee system proposed here did not increase the abundance and richness of wasps. Predatory wasps likely depend on the presence of non-agricultural habitats to find all the necessary resources and nesting sites not available on monocultures (Pereira et al., 2007). However, they need to continually move between nests and feeding habitats (Richter, 2000). The genus *Polybia* spp., for instance, has a foraging range of 75-126 m from its nest (Bichara-filho et al., 2010; Prezoto; Gobbi, 2005; Santos et al., 2000).

The distance between our implanted systems to any forest in the surroundings involves a radius of more than 1 km. This may have hampered the initial attractiveness of a greater number of wasps than those that already inhabit close to the implanted coffee systems. Studies indicate that predatory wasps prefer trees as nesting sites, favoring their permanence in woody systems (Santos; Bispo; Aguiar, 2009; Souza et al., 2014). Therefore, the density of the implanted system seems not yet sufficient for wasps to feed and at the same time to nest.

The parasitoids emerged from CLM belongs to Eulophidae and Braconidae families, both are reported to parasitize CLM (Pereira et al., 2007; Lomeli-Flores et al., 2009). We also found these families visiting the introduced plants in the diversified coffee system. We suggest that the nectar of *I. edulis*, *S. macranthera* and *V. curassavica* added to nectar of non-crop plants is the main responsible for the increase of the parasitoid population in diversified coffee system. Besides, our results showed that the parasitism rate of CLM was higher in diversified coffee systems than conventional. These results allow us to conclude that the proposed diversified system is capable of sustaining parasitoid populations of CLM and increasing these natural enemy populations. Other studies showed that mortality caused by parasitoids may range from 10% to 27% of CLM population (Lomeli-Flores et al., 2009; Rezende et al., 2014). Thus, we found a parasitism rate apparently low compared to other works carried out in other regions (Pereira et al., 2007; Rezende et al., 2014). Here we observed 8% rate of parasitism on CLM in diversified coffee systems and 6% in conventional. Studying the association of inga trees in coffee crops Rezende et al. (2014) observed more than 30 % of parasitism rate and more than 20% in coffee monocultures. Therefore, we suggest that sites that already present a complex landscape when added more complexity, provide even more control of CLM by parasitoids then a diversified system implanted recently inside of extensive monocultures areas. Finally, we showed that the diversified coffee systems showed higher parasitism rate of CLM.

Despite the higher parasitism rate of CLM found in the diversified system, there was no difference in CLM infestation, comparing to conventional coffee systems in 2019 and 2021 and it 2020 was higher. Even though they did not reach the economic threshold, 20% of infestation by CLM (Reis and Souza, 1996). Therefore, since the CLM control level has not been reached, spending on insecticides to control of CLM was unnecessary. This happened in the conventional systems in this study, which followed all the agronomic practices regularly used in the Cerrado region. In addition to unnecessary expenses, these environmental practices contaminate the environment and the damage is immeasurable.

The rust infestation in our experimental plots show that control measures for this disease must be taken into account before implementing any systems that involve the removal of fungicides. Here, we initially removed the fungicide application in the diversified system in the first year after the installation of the experiment. In 2020 and 2021 we used an alternative control with copper and hydrogen peroxide aiming to control the rust. Although the attack by rust has increased over time but decreased in diversified system compared to the conventional ones, the use of alternatives to control the disease must be carried out from the beginning. Another possibility is the implementation of diversified systems in coffee crops where the plants are resistant to rust as “Catiguá MG 1, MG 2 and MG 3” (Zambolim, 2016) and “MGS Paraíso 2” which is recommended for the Cerrado region.

The diversification in agroecosystems have the potential of improve pest control by increasing natural enemy populations (Jezeer et al., 2018; Muschler, 2001; Somporn et al., 2012; Vaast et al., 2006; Rezende et al., 2021). Here, our study shows that there were no signicative atack by CLM when pesticides were withdrawn from the strategically diversified crop. This result is especially important if we take into account the economic expenses of pesticides e their spraying, the labor involver for application, environmental contamination and possible human intoxications. This highlights the importance of management strategies that seek to combine cost reduction with the operationalization of processes, the reduction of risks to workers and consumers, and to the environment. Moreover, it adds value to the final product produced without pesticides. In this sense, our results show that the strategic association with I. edulis, S. macranthera, V. curassavica and non-crop plants with coffee crops can be used in order to increment CLM management with more safety for the environment and humans, and maintaining biodiversity in coffee agroecosystems. Several ecosystem services can benefit from the diversification of the agricultural landscape, such as carbon sequestration and nutrient cycling (Venzon et al., 2021). Therefore, a higher density of associated plants to coffee crops can be also promoting additional ecosystem services mitigating the effects of conventional farming.

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Tables

Table 1. Abundance of insects collected on extrafloral nectaries of *I. edulis* and *S. macranthera* and on inflorescence of *V. curassavica* in diversified coffee plots in Patrocínio-MG.

		Associated plant species			
Families	**Insects Species**	***Inga edulis***	***Senna macranthera***	***Varronia curassavica***	**Total**
Formicidae	*Brachymyrmex* sp.1	72	25	27	124
	Brachymyrmex sp.2	12	3	7	22
	Brachymyrmex sp.3	1	2	-	3
	Camponotus sp.1	2	-	1	3
	Cardiocondyla sp.1	4	-	-	4
	Dorymyrmex sp.1	36	10	14	60
	Linepithema sp.1	23	6	32	61
	Pheidole sp.9	9	5	1	15
	Pseudomyrmex sp.1	-	-	1	1
	Pseudomyrmex sp.2	1	1	-	2
	Solenopsis saevissima	6	-	11	17
Coccinellidae	*Harmonia axyridis*	-	1	4	5
Chrysomelidae	sp.1	-	-	52	52
Melyridae	sp.1	-	-	17	17
Scarabaeidae	sp.1	-	-	1	1
Telegeusidae	sp.1	1	-	1	2
Otitidae	sp.1	3	3	-	6
Sarcophagidae	sp.1	1	-	3	4
Syrphidae	sp.1	-	1	3	4
Tachinidae	sp.1	-	-	3	3
Tephritidae	sp.1	-	2	2	4
Vespidae	*Mischocyttarus drewseni*	-	-	1	1
	Polybia occidentalis	-	1	1	2
Apidae	*Apis mellifera*	-	-	16	16
	Trigona spinipes	-	-	2	2
Braconidae	sp.1	5	5	11	21
Eulophidae	sp.1	1	1	1	3
	Proacrias coffeae	-	-	2	2
Mymaridae	sp.1	1	-	-	1
Anthocoridae	*Orius insidiosus*	-	-	2	2
Aphididae	sp.1	2	1	-	3
Largidae	sp.1	-	-	1	1
Lygaeidae	sp.1	-	-	3	3
Lyonetiidae	*Leucoptera coffeella*	2	1	-	3
Miridae	sp.1	-	-	2	2
Pentatomidae	sp.1	-	-	2	2
Pyrrhocoridae	sp.1	-	-	2	2
Reduviidae	sp.1	-	-	1	1
Scuteleridae	sp.1	-	-	1	1
Total		182	68	229	479

Table 2. Abundance of green lacewings species in diversified and conventional coffee systems in Patrocínio-MG.

Chrysopidae species	**Diversified coffee system**	**Conventional coffee system**	**Total**
Ceraeochrysa cincta	34	32	66
Ceraeochrysa everes	4	1	5
Ceraeochrysa sanchezi	2	1	3
Chrysoperla externa	6	2	8
Chrysopodes divisus	31	20	51
Total	77	56	133

(χ^2=2.1996; DF=1; p=0.13805)

Table 3. Abundance of predatory wasp species collected in diversified and conventional coffee systems in Patrocínio-MG.

Vespidae species	**Diversified coffee system**	**Conventional coffee system**	**Total**
Agelaia multipicta	3	3	6
Agelaia pallipes	6	0	6
Polybia ignobilis	2	1	3
Polybia occidentalis	1	2	3
Polybia platycephala	1	4	5
Polybia sericea	4	-	4
Total	17	10	27

(χ^2=1.7728; DF=1; p=0.183033)

Table 4. Abundance of coffee leaf miner parasitoids in diversified and conventional coffee systems in Patrocínio-MG.

Parasitoid species	**Diversified coffee system**	**Conventional coffee system**	**Total**
Closteroceus sp.	4	6	10
Cirrospilus sp.	3	1	4
Horismenus sp.	2	1	3
Orgilus niger	44	23	67
Proacrias coffeae	105	108	213
Stiropius reticulatus	35	9	44
(Undentified)	33	15	48
Total	226	163	389

Table 5. Mean of active mines, inactive mines, preyed mines and parasitized mines in coffee leaves in conventional and diversified coffee system in Patrocínio-MG.

Parameter	**Year**	**Diversified coffee system (mean ± se)**	**Conventional coffee system (mean ± se)**	**p-value**
Active mines	2019	0.031 ± 0.009	0.382 ± 0.011	0.099^{ns}
	2020	3.090 ± 0.009	1.875 ± 0.007	0.002*
	2021	2.662 ± 0.011	2.083 ± 0.009	0.260^{ns}
Inactive mines	2019	0.066 ± 0.009	0.128 ± 0.011	<0.05*
	2020	6.701 ± 0.013	6.111 ± 0.012	0.276^{ns}
	2021	10.30 ± 0.022	6.307 ± 0.017	<0.05*
Preyed mines	2019 2020 2021	0.009 ± 0.001	0.013 ± 0.001	0.777^{ns}
Rust	2019	0.147 ± 0.026	0.074 ± 0.017	<0.05*
	2020	12.53 ± 0.027	6.84 ± 0.0192	<0.05*
	2021	19.61 ± 0.0354	29.74 ± 0.043	<0.05*

Means (± SE) of parameters for each treatment compared by χ^2 test ($p<0.05$)

Figures

Figure 1. *Inga edulis* introduced into coffee crops at Experimental area at EPAMIG's Farm in Patrocínio-MG. Highlight: composite leaf of *I. edulis* with their extrafloral nectaries (Photo: Elem Fialho Martins).

Figure 2. *Senna macranthera* introduced into coffee crops at Experimental area at EPAMIG's Farm in Patrocínio-MG. Highlight: composite leaf of *S. macranthera* with extrafloral nectary. (Photo: Jualiana Maria de Oliveira).

Figure 3. *Varronia curassavica* introduced into coffee crops at Experimental area at EPAMIG's Farm in Patrocínio-MG. Highlight: inflorescence of *V. curassavica* (Photo: Elem Fialho Martins).

Figure 4. Experimental area at EPAMIG's Farm in Patrocínio-MG. Red squares represent the diversified coffee systems and yellow squares represent the conventional coffee systems.

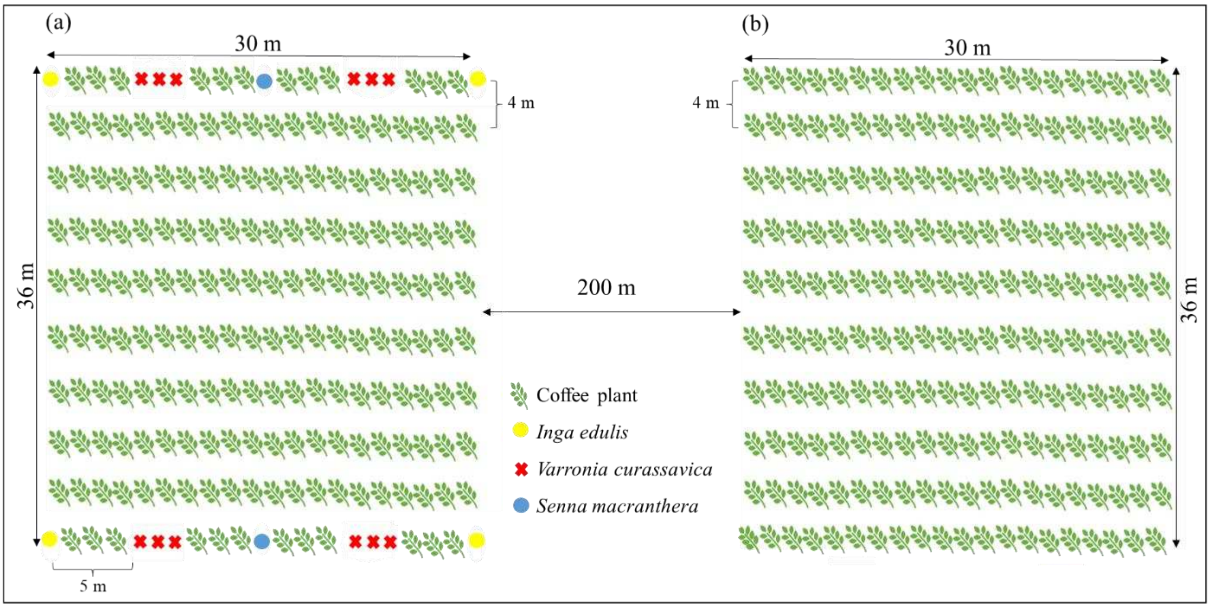

Figure 5. Diversified coffee system plot (a) and conventional coffee system plot (b). Area of each plot = 1,080 m².

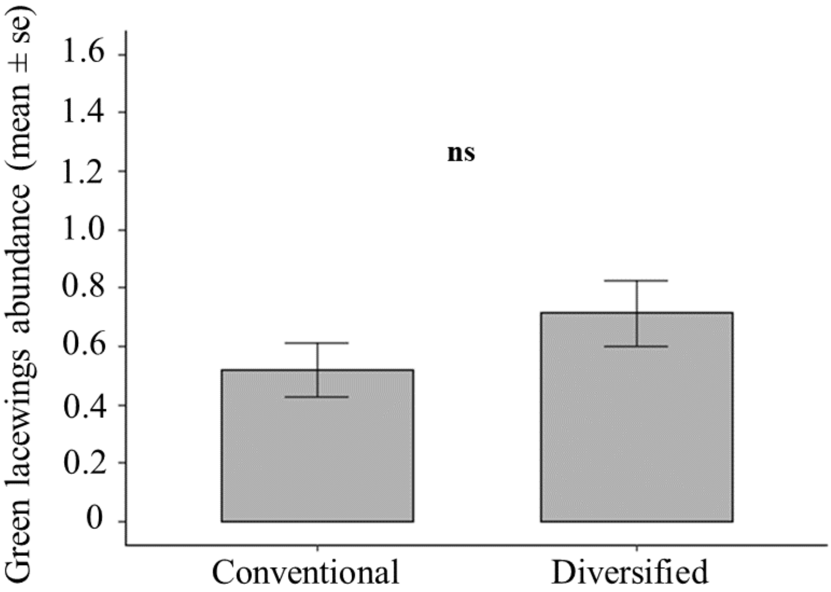

Figure 6. Green lacewings abundance in conventional and diversified coffee systems (χ^2= 2.1996; df=1; p=0.13805).

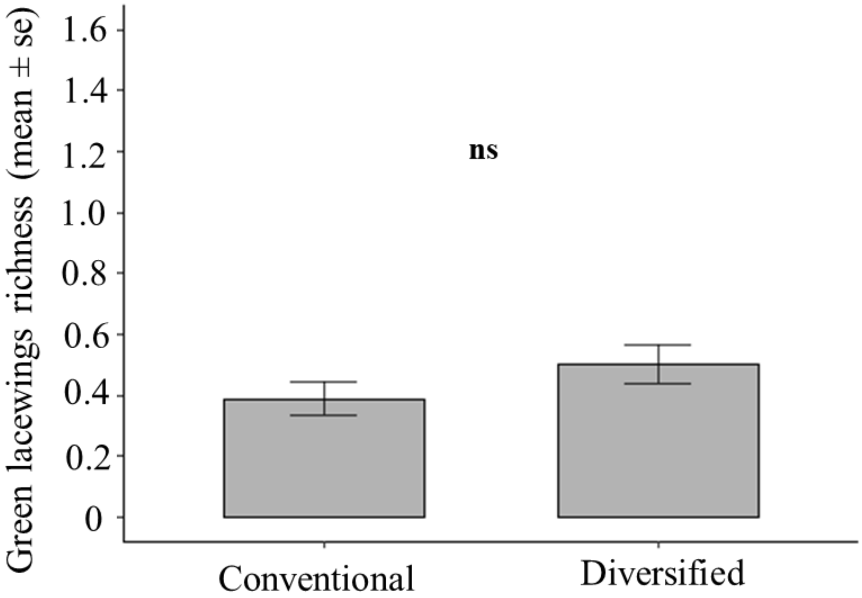

Figure 7. Green lacewings richness in conventional and diversified coffee systems (χ^2=1.4921; df=1; p=0.2219).

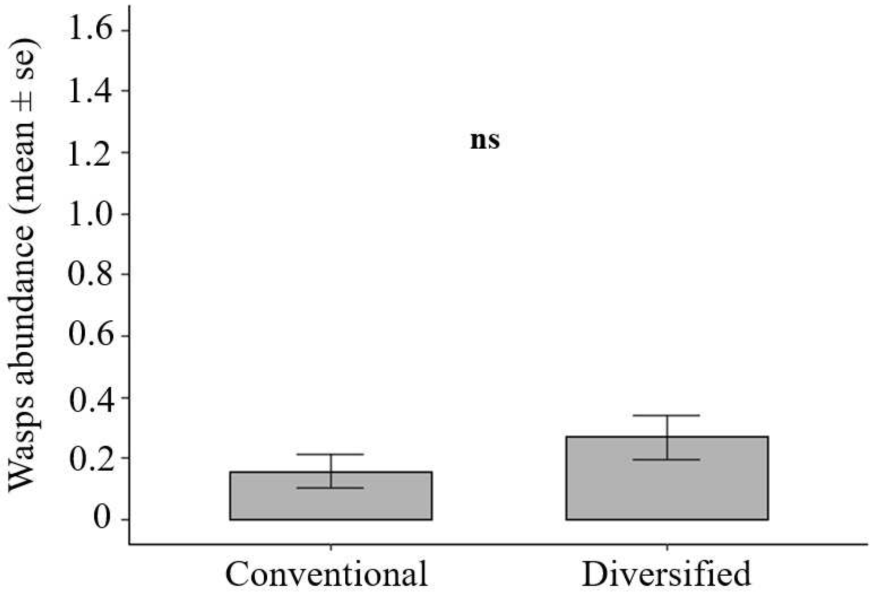

Figure 8. Wasps abundance in conventional and diversified coffee systems (χ^2=1.7728; df=1; p=0.183033).

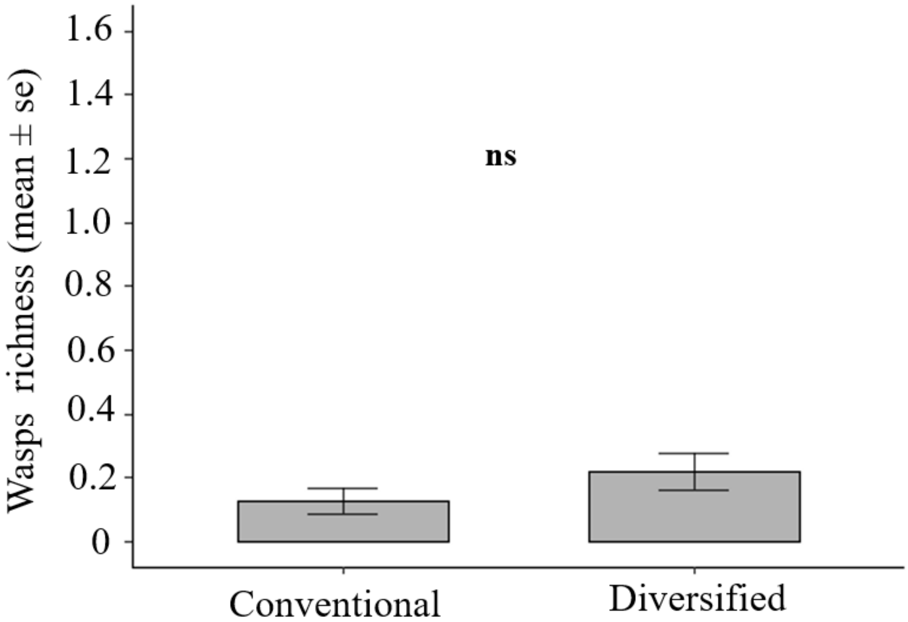

Figure 9. Wasps richness in conventional and diversified coffee systems (χ^2=1.5943; df=1; p=0.2067).

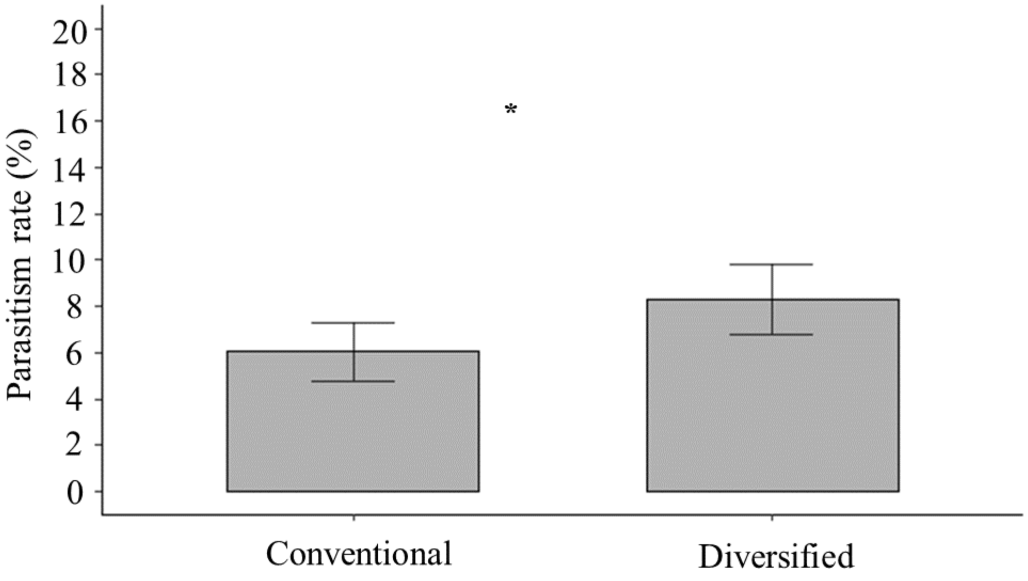

Figure 10. Parasitism rate of coffee leaf miner in conventional and diversified coffee systems in 2019, 2020 and 2021 (z=-4.646; df=1; p= 0.00000338).

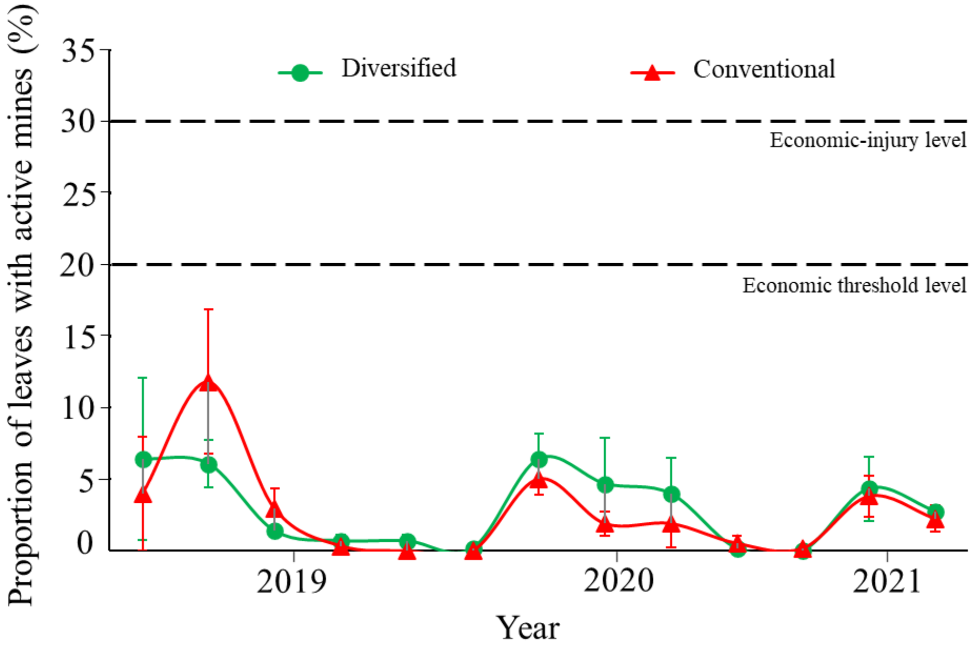

Figure 11. Infestation by coffee leaf miner in diversified and conventional coffee systems in 2019 2020 and 2021.

Supplementary Material

Appendix 1. Plant Nutrition (soil fertilization, coffee husk compost and foliar fertilization) in the plots with diversified and conventional coffee systems in a conventional and diversified coffee system in Patrocínio-MG, Brazil.

Soil fertilization

Year	Month	Diversified coffee system	Conventional coffee system
2018	Dec	Urea (750 kg/ha)	Urea (750 kg/ha)
2019	Feb	NPK 20-05-20 (500 kg/ha)	NPK 20-05-20 (500 kg/ha)
	Oct	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	Urea (300 kg/ha)	Urea (300 kg/ha)
	Jan	NPK 20-05-20 (500 kg/ha)	NPK 20-05-20 (500 kg/ha)
2020	Mar	NPK 20-00-20 (400 kg/ha)	NPK 20-00-20 (400 kg/ha)
	Nov	NPK 20-05-20 (500 kg/ha)	NPK 20-05-20 (500 kg/ha)
	Dec	Simple superphosphate (500 kg/ha)	Simple superphosphate (500 kg/ha)
2021	Feb	NPK 20-05-20 (500 kg/ha)	NPK 20-05-20 (500 kg/ha)

Coffee husk compost

Year	Month	Diversified coffee system	Conventional coffee system
2018	Dec	5 kg/linear meter	5 kg/linear meter

Foliar fertilization

Year	Month	Diversified coffee system	Conventional coffee system
2018	Dec	Potassium (10%), magnesium (2%), sulfur (8.26%), boron (6%), manganese (8%), molybdenum (0.10%) and zinc (3%) (5kg/ha)	Potassium (10%), magnesium (2%), sulfur (8.26%), boron (6%), manganese (8%), molybdenum (0.10%) and zinc (3%) (5kg/ha)
2019	Feb		
	Dec		-----
2020	Feb	-----	Potassium (10%), magnesium (2%), sulfur (8.26%), boron (6%), manganese (8%), molybdenum (0.10%) and zinc (3%) (5kg/ha)
	Mar	Potassium (10%), magnesium (2%), sulfur (8.26%), boron (6%), manganese (8%), molybdenum (0.10%) and zinc (3%) (5kg/ha)	-----
2021	Jan	Nitrogen (10%), boron (1%), manganese (4%), copper (0.5%) and zinc (6%) (2 l/ha)	Nitrogen (10%), boron (1%), manganese (4%), copper (0.5%) and zinc (6%) (2 l/ha)
	Apr		

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

Insecticide

Year	Month	Diversified coffee system	Conventional coffee system
2019	Feb	-----	Curbix® (2.5 l/ha)
2020	Feb		
2021	Jan		
	Apr		

Insecticide /Acaricide

Year	Month	Diversified coffee system	Conventional coffee system
2018	Dec	-----	avermectin (0.4 l/ha)
2019	Feb		
2020	Feb		
2021	Jan		Abamectin: avermectin (0.4 l/ha)
	Apr		

Fungicide

Year	Month	Diversified coffee system	Conventional coffee system
2018	Dec	-----	Pyraclostrobin: strobilurin + epoxiconazole: triazole (1.5 l/ha) Copper hydroxide: inorganic (1.5 kg/ha)
2019	Feb		
2020	Feb	-----	Pyraclostrobin: strobilurin + epoxiconazole: triazole (1.5 l/ha) Copper hydroxide: inorganic (1.5 kg/ha)
2020	Mar	Copper hydroxide: inorganic (1.5 kg/ha)	-----
2021	Jan	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)
	Apr		

Insecticide/Fungicide

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

Sodium hypochlorite

Year	Month	Diversified coffee system	Conventional coffee system
2019	Dec	Solution 1%	-----
2020	Mar		
2021	Jan		
	Apr		

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

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

Herbicide[1]

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

Non-crop plants mechanic suppression

Year	Month	Diversified coffee system	Conventional coffee system
2019	May	Suppressed up to 10 cm	Suppressed up to 10 cm
2020	Jan	Suppressed up to 50 cm	
2020	Mar		
2020	Jun	Suppressed up to 10 cm	
2020	Dec	Suppressed up to 50 cm	
2021	Mar		

Non-crop plants manual suppression[2]

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

[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 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	0.41 L	36.00/L	14,76	2	-
Avermectin	0.41 L	45.00/L	18,45	3	-
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	-
Insecticides/Fungicides					
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			1388,87		270,00

GENERAL CONCLUSIONS

The green lacewing *Ceraeochrysa cubana* successfully prey on the coffee leaf miner *Leucoptera coffeella* eggs and pupae. It is the first time that a Chrysopidae species is reported as predator of the pest eggs. Therefore, we add a new Chrysopidae species to the list of natural enemies of coffee leaf miner.

Larvae of *C. cubana* survive longer in the presence of *V. curassavica* inflorescences, and decrease the population growth rate of coffee leaf miner.

Coffee crop diversification with *Inga edulis*, *Senna macranthera*, *Varronia curassavica* and non-crop plants, in the absence of pesticides, did not affect populations of green lacewings and wasps, but did increase parasitoids and its parasitism rate do coffee leaf miner. Coffee leaf miner populations did not vary significantly among diversified and monoculture coffee crops, but they did not reach economic threshold.

Therefore, this study can be used as a basis to new research's that involves of coffee diversification and in the same time can be used by farmers aiming the management of CLM in coffee crops.