DANIEL MACIEL MARQUES

INTERCROPPING COFFEE WITH *VARRONIA CURASSAVICA* JACQ. AFFECTS ARTHROPOD BIODIVERSITY

Dissertation submitted to the Entomology Post Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Madelaine Venzon

Co-advisers: Maira Christina Marques Fonseca
Elem Fialho Martins
Rodrigo Cupertino Bernardes
Emerson Ferreira Vilela

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Assent:

Documento assinado digitalmente
DANIEL MACIEL MARQUES
Data: 26/10/2023 12:31:10-0300
Verifique em https://validar.iti.gov.br

Daniel Maciel Marques
Author

Documento assinado digitalmente
MADELAINE VENZON
Data: 26/10/2023 12:13:55-0300
Verifique em https://validar.iti.gov.br

Madelaine Venzon
Adviser

To my parents, friends, colleagues and me.

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“Shall We Begin?”
(Daenerys Targeryan)

ABSTRACT

MARQUES, Daniel Maciel, M.Sc., Universidade Federal de Viçosa, July, 2023. **Intercropping coffee with *Varronia curassavica* Jacq. affects arthropod biodiversity** Adviser: Madelaine Venzon Co-advisers: Maira Christina Marques Fonseca, Elem Fialho Martins, Rodrigo Cupertino Bernardes and Emerson Ferreira Vilela.

Coffee is one of the most important commodities in Brazil, and the country is the largest producer of coffee berries in the world. The changes in the global perception of the importance of agricultural landscapes, climate change, contamination by pesticides and loss of biodiversity haves led to improve the research and the practices on more sustainable agroecosystem and food production. In this sense, companion crop plants can be powerful allies to promote sustainable agroecossystems, reducing the effects of climate changes and loss of biodiversity, as well as improving the natural pest control by offering plant resources needed by natural enemies, resulting in less crop damage by pests. The objective of this study was to evaluate the potential of *Varronia curassavica* as companion crop to improve pest control of coffee leafminer (CLM) and coffee berry borer (CBB) and to increase the biodiversity, especially parasitoids and predators, as well as studying its protection to coffee plants. The first experiment was carried out in a coffee farm in Paula Cândido, in the Atlantic Forest biome, and the second in Patrocínio, in the Cerrado biome, both in the state of Minas Gerais, Brazil. At Paula Cândido, we sampled coffee plants for CLM and CBB from January and May of 2023. Biodiversity was also evaluated by sampling arthropods on coffee trees using entomological net and by tray beating. The effect of distance on the protection of coffee plants against CLM provided by *V. curassavica* was evaluate on transects in a farm where *V. currassavica* was associated to coffee in Patrocinio. No differences in infestation levels in diversified and conventional plots were observed in Paula Cândido, which was low probably due to chemical inputs and the rainy season. The total number of natural enemies sampled was not significant different in both plots, but diversified plots showed significant higher abundance of predators of Geocoridae and Linyphiidae families and parasitoids of Tachinidae and Chalcididae families. The mean infestation rate of *Leucoptera coffeella* on the diversified system in Cerrado was low and no differences were observed on the infestation levels across the transect. These results show that *Varronia curassavica* can be a suitable plant to be used in agroecossystems

Keywords: Coffee leafminer. Coffee berry borer. Natural enemies. Biodiversity.

RESUMO

Marques, Daniel Maciel, M.Sc., Universidade Federal de Viçosa, julho de 2023. **O consórcio do café com *Varronia curassavica* Jacq. afeta a biodiversidade de artrópodes**. Orientador: Madelaine Venzon. Coorientadores: Maira Christina Marques Fonseca, Elem Fialho Martins, Rodrigo Cupertino Bernardes e Emerson Ferreira Vilela.

O café é uma das commodities mais importantes no Brasil, e o país é o maior produtor de grãos de café do mundo. As mudanças na percepção global da importância das paisagens agrícolas, as mudanças climáticas, a contaminação por pesticidas e a perda de biodiversidade levaram ao aumento nas pesquisas e nas práticas de agroecossistemas e produção de alimentos mais sustentáveis. Nesse sentido, plantas companheiras podem ser aliadas poderosas para promover agroecossistemas sustentáveis, reduzindo os efeitos das mudanças climáticas e da perda de biodiversidade, bem como melhorando o controle natural de pragas, ao oferecer recursos vegetais necessários aos inimigos naturais. O objetivo deste estudo foi avaliar o potencial da *Varronia curassavica* como planta companheira para melhorar o controle de pragas do bicho-mineiro-do-café (CLM) e da broca-do-café (CBB), aumentar a biodiversidade, especialmente de parasitoides e predadores, além de estudar sua proteção às plantas de café ao longo e um transecto. O primeiro experimento foi realizado em uma fazenda de café em Paula Cândido, no bioma da Mata Atlântica, e o segundo em Patrocínio, no bioma do Cerrado, ambos no estado de Minas Gerais, Brasil. Em Paula Cândido, amostramos plantas de café para CLM e CBB de janeiro a maio de 2023. A biodiversidade também foi avaliada por meio da amostragem de artrópodes no café usando rede entomológica e batida de bandeja. O efeito da distância na proteção das plantas de café contra CBB, proporcionada por V. curassavica, foi avaliado em transectos em uma fazenda onde *V. curassavica* estava associada ao café em Patrocínio. Em Paula Cândido, não foram observadas diferenças nos níveis de infestação entre as parcelas diversificadas e convencionais, que estavam baixos, provavelmente devido aos insumos químicos e à estação chuvosa. O número total de inimigos naturais nas parcelas diversificadas não apresentou diferença significativa em relação ao convencional, mas tiveram maior abundância de predadores das famílias Geocoridae e Linyphiidae e parasitoides das famílias Tachinidae e Chalcididae. No Cerrado, o nível de infestação de CLM estava baixo e não foram observadas diferenças nos níveis de infestação ao longo do transecto. Esses resultados mostram que *V. curassavica* pode ser uma planta adequada para ser usada em agroecossistemas.

Palavras-chave: Bicho mineiro do café. Broca do café. Inimigos naturais. Biodiversidade.

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SUMMARY

1. INTRODUCTION

Coffee, one of the most consumed beverages worldwide, is an important commodity produced in Brazil, both culturally, often regarded as a beloved morning ritual, and economically, as the country is the world's largest producer. In 2022, Brazil produced about 2 millions of tons of coffee beans, with the state of Minas Gerais being the major producer in the country (Volsi et al, 2019; Our World in Data, 2023; IBGE, 2016; ABIC, 2023). Regarding to the production, coffee has traditionally followed conventional agricultural and monocropping practices that aiming to the high productivity. However, this conventional system leads to environmental degradation by the heavily reliance on chemical inputs like pesticides, herbicides and synthetic fertilizers. Together with the simplification of the landscape by growing a single crop in large areas, these factors cause the death and loss of habitat of non-target organisms like pollinators and other beneficial organisms, biodiversity loss, ecological imbalance and contribute to climate change (Ibanez and Blackman, 2016; Foley et al, 2005; Tillman et al, 2002). Furthermore, it is known that chemicals pesticides and their residues can accumulate in the environment and even in the animal tissues and pollute water and soil, posing a risk of contamination to farmers, consumers and nature (Karami-Mohajeri and Abdollahi, 2010; Chopra, Sharma and Chamoli, 2011).

In the last years, however, a new approach has risen: the agroecology. It is an agricultural model that comes to solve the problems that conventional agricultural has left, many of them already mentioned above (Wezel et al, 2009). The purpose of agroecology systems is to provide a sustainable food production by developing environmentally friendly products and methods that can substitute, minimize or avoid the use of chemical inputs and mitigate the effects of climate changes, among other benefits. Another goal of the agroecology is to regenerate the ecological balance in agricultural landscapes by adopting some practices, like protecting the soil from erosion and degeneration, improving the organic matter, using crop rotation, reducing tillage and using cover crops (Wezel et al, 2009; Altieri, 2018). The use of cover crops and companion plants is also important to create an agroecosystem that helps build resilience in agricultural communities and ecosystems, reducing vulnerability to climate-related risks. These diversified environments improve the biodiversity on agricultural ecosystems, creating and recovering habitats lost in the monoculture systems, enhancing the ecosystems services provided by organisms like pollinators and natural enemies and improving the natural control of major pests of the crops (Kremen and Miles, 2012; Venzon 2021). All these methods, when used correctly and scientific-based, can help to mitigate the effects of climate changes by preventing deforestation of new areas to grow crop plants by keeping the health of soil, avoid losses of biodiversity, improve the ecological services provided by local species (Kremen and Miles, 2012; Bommarco, Kleijn and Potts, 2013; Borma et al, 2022).

Plant diversification is also a strategy used in the conservative biological control (CBC), also known as conservation biological control, which is an environmentally friendly approach to managing pest populations in agricultural ecosystems through modification of the environment thereby reducing the use of pesticides (Barbosa, 1998; Jonson et al, 2007). This strategy of controlling pests relies on the existing populations of beneficial organisms such as predators, parasitoids, and pathogens that already exist in the agricultural landscapes by providing them suitable habitat, shelter and food (Barbosa, 1998; Venzon, 2021; Blassioli-Moraes et al, 2022). The CBC is a promising approach to control pests, however, it requires a good knowledge of the ecology and interactions of the natural enemies and their prey to understand and maximize the results observed in the field (Jonsson et al, 2007; Venzon and Sujii, 2009).

In coffee farms, conservation biological control can play a crucial role in achieving a more sustainable production system (Rezende et al, 2014; Rezende et al, 2021; Rosado et al, 2021; Venzon, 2021). Natural enemies such as parasitoid wasps, predators, and entomopathogenic fungi have the potential to control the two major coffee pests, the coffee leaf miner (CLM), *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842), and the coffee berry borer (CBB), *Hypothenemus hampeii* (Ferrari 1867) (Lomeli-Flores, Barrera and Bernal, 2009; Escobar-Ramírez et al, 2019; Morris et al 2018; Venzon, 2021). By relying on these natural enemies, farmers can reduce their dependency on pesticides and minimize the associated negative impacts. Furthermore, coffee plants naturally harbor a diverse range of these natural enemies, but, in monoculture farms, their numbers are often insufficient to effectively suppress populations of CBB and CLM, necessitating the implementation of conservation biological control measures (Fernandes et al, 2008; Lomeli-Flores, Barrera and Bernal, 2009; Venzon, 2021).

To enhance the implementation of conservative biological control strategies and promote natural pest population regulation, it is imperative to prioritize crop diversification within coffee farms (Venzon, 2021). Companion plants play a pivotal role by offering resources such as nectar, pollen and shelter through all the year and favorable microclimate conditions to survivorship of the natural enemies (Redlich, Martin and Steffan-Dewenter, 2018). These companion plants encompass a variety of options, including trees, cover crops, and non-cropping plants that can be strategically positioned or kept in the surrounding areas or even within intercropping. Moreover, monocultures provide an ideal environment for the coffee berry borer and coffee leaf miner due to their monophagous feeding habits (Le Pelley, 1968). Unlike their predators and parasitoids, which needs a wider range of resources either during a non-carnivore life stage, or to complement or supplement they prey diet, these pests primarily rely on the berries and leaves of coffee plants, respectively, to complete their life cycles, breed, and thereby causing damage. Thus, it is essential to conduct studies that assess potential plant species that are suitable for use as companion plants. These investigations will contribute to enhancing the effectiveness of conservation biological control strategies, ensuring that they are supported by scientifically grounded results. By identifying and incorporating appropriate companion plants, coffee farms can create an environment that fosters the presence and activity of natural enemies, ultimately promoting sustainable pest management practices (Venzon, 2021).

One potential companion plant that can be beneficial in agroecosystems is *Varronia curassavica* Jacq, a member of the Boraginaceae family. It is a perennial plant native to South and Central America, found in diverse biomes such as the Atlantic Forest, sandy soils, and restinga areas (Marques et al., 2019). This shrub can grow up to 2 meters in height and it is characterized by a branched stem, sessile leaves, dense inflorescence with white flowers, and red fruits (Feijó et al., 2014). One notable characteristic of *V. curassavica* is its ability to attract a wide variety of insect species and keep the formation of inflorescence and fruits by a long period throughout the year (Brandão et al., 2015, Martins, 2017). Moreover, the essential oil derived from *V. curassavica* possesses various properties, including anti-inflammatory and analgesic effects, as well as antimicrobial and insecticidal effects against certain crop pests (Matias et al., 2013; Andrade et al., 2021). Some studies carried out at Cerrado biome evaluated the potential of *V. curassavica* together with other plants as intercropped plants in coffee crops and found that diversification can improve the biological control of coffee pests (Botti, 2021; Franzin, 2021; Ferla et al, 2023). However, information about the density of *V. currassavica* plants and its action ray to protect the crops on farm is missing. For instance, Rezende et al, (2021), found the *Inga* tress have the potential to protect coffee crops against CLM e CBB in a ray of 20 meters. Moreover, any study contemplated the association of *V. currassavica* and coffee at Atlantic Forest biome, especially the Zona da Mata Mineira, which is an important producer region with a production area of 6.141 acres and about 7.105 coffee bags produced in 2022 (ABIC, 2023).

The aim of this study is to assess the potential of *V. curassavica* as a companion plant in a conventional coffee farm at Zona da Mata, investigating whether the presence of *V. curassavica* can effectively reduce infestations levels of the CBC and CLM and evaluating its capacity to improve biodiversity, particularly by enhancing the populations of natural enemies. Additionally, we also evaluate the action ray provided by *V. curassavica* against infestation level of CLM to coffee plants at Cerrado.

2 MATERIALS AND METHODS

2.1. Experiment conducted in a coffee farm at Zona da Mata Mineira

The coffee farm is located at Paula Cândido, Minas Gerais, Brazil (20°49'36.44"S, 42°55'0.27"W, altitude 721 meters). The species of coffee plant cultivated is *Coffea arabica*, Catuaí cultivar, in a conventional system, with the use of fertilizers, herbicides and pesticides. The area is in the Atlantic Forest biome. The study area has about 2.44 hectares and was divided in eight plots of 0.15 hectare each, with a distance of 25 meters from each other (Figures 1, 2). Four plots were maintained as monoculture unmodified control, while the other four plots we added potted *V. curassavica* (Figure 2). Seeds of *V. curassavica* were obtained at Campo Experimental de Oratórios, from Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG) and the potted plants were about two years old with height between 30 and 1.20 meters. The plant pots used have 11 liters and were filled with sandy and soil substrate. We identified the plots according with treatment: monoculture/control were classified as conventional and plots with *V. curassavica* potted plants were classified as diversified. The samples started one week after placing the plants in the farm.

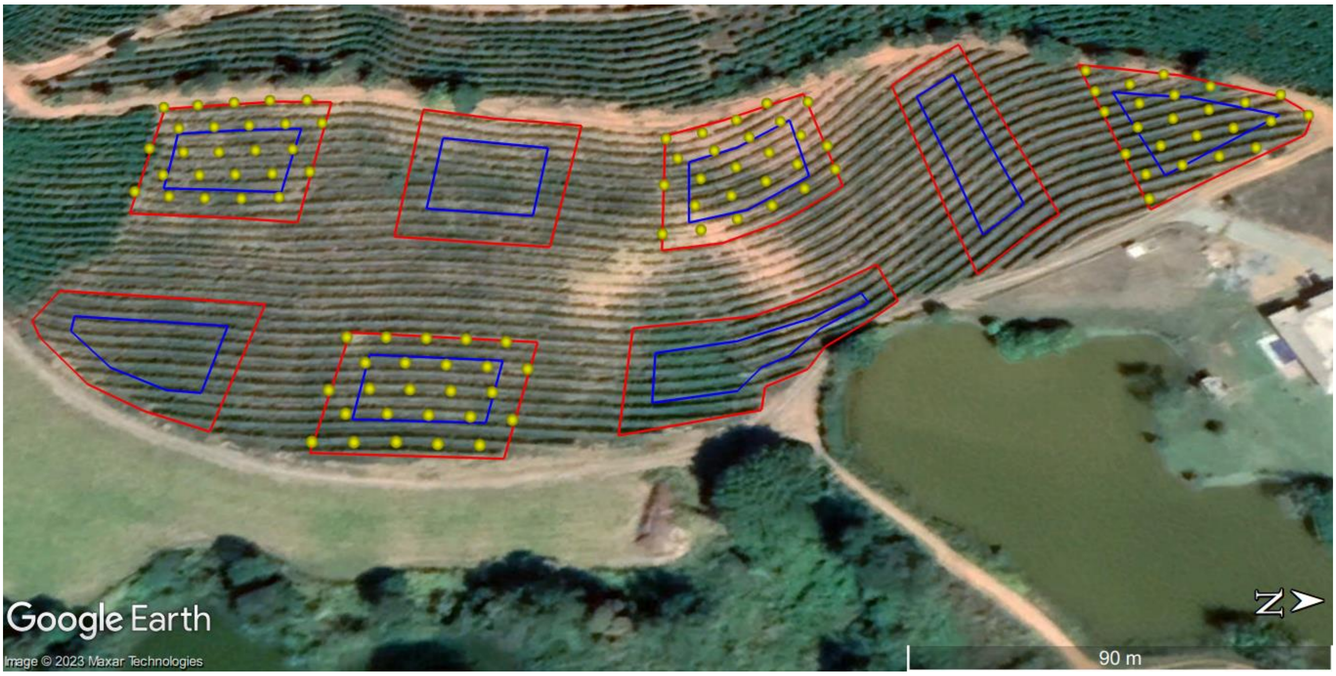

Figure 1. Experimental area. Red lines are delimitations of plots; blues lines are delimitations of sampling areas; yellow dots are potted *Varronia curassavica* plants. Image obtained with software Google Earth Pro 7.3.6.9345.

The plots used as control and as diversified were randomly selected in the experimental area. Twenty-five *V. curassavica* potted plants were added in each diversified plot. They were distributed each 10 meters in the same coffee line and, leaving one coffee line with *V. curassavica* and one without (Figure 1, 2). The samplings occurred only in the useful plots, the area located in the middle of each parcel, in order to avoid interference of the surrounding environment (Figure 1, 2). All samplings described below occurred each 15 days between January and May from 2023 at Zona da Mata Mineira. In total, we carried out seven sampling dates. We measured the damage caused by two major coffee pests, the CLM and CBC. For the CLM, ten coffee plants were assessed in each plot, and eight leaves were collected from each plant, specifically, the fourth pair of leaves on the branches in each cardinal direction (north, south, west, and east).

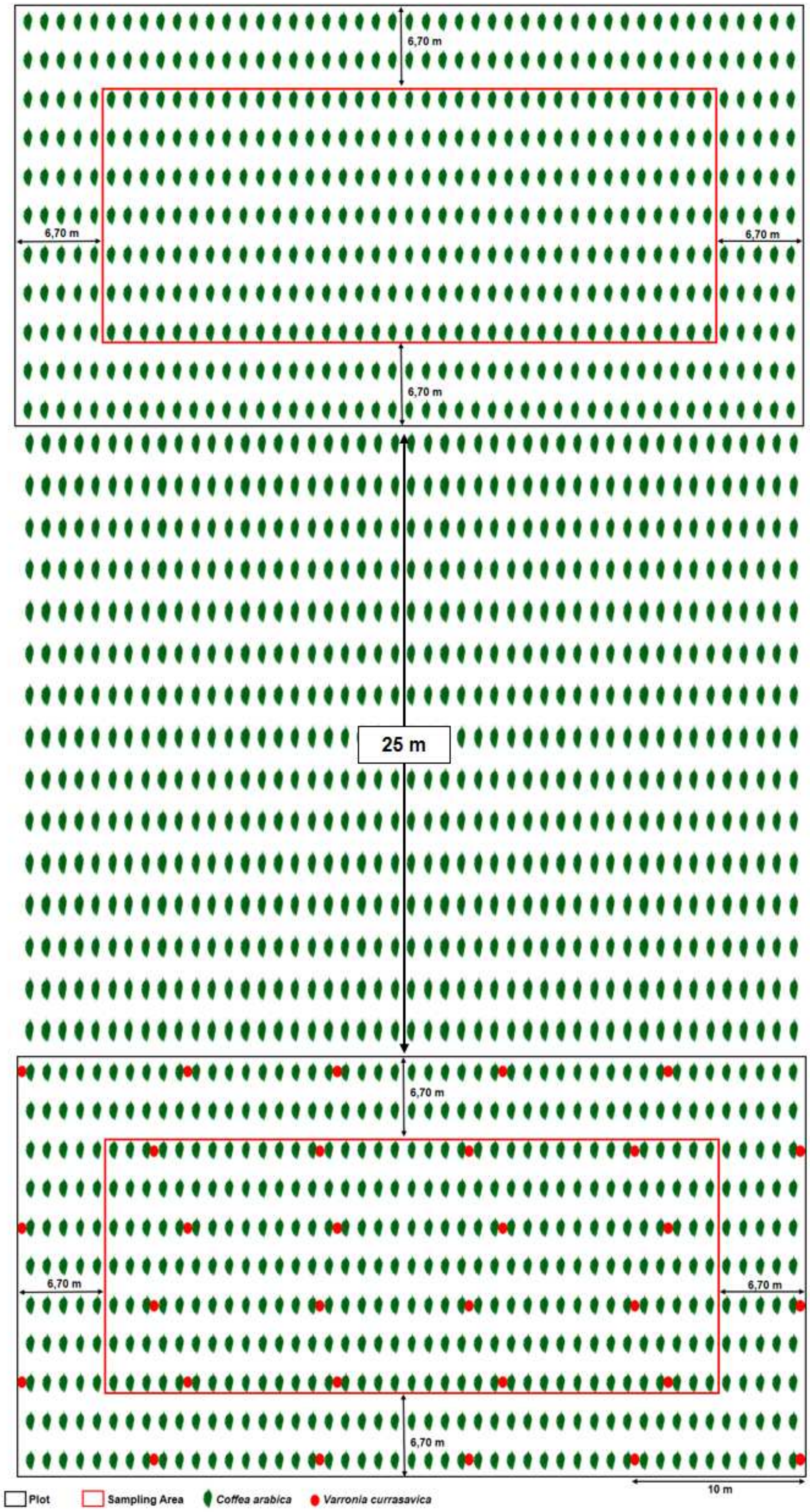

Figure 2. Schematic design of the experimental area indicating the measurements. The plot above is monoculture and the bellow is the diversified with *V. curassavica*.

Subsequently, the collected leaves were analyzed in the laboratory to determine the proportion of mined leaves. Regarding the CBB, the same plants assessed for CLM were evaluated and fifty coffee berries were examined in the field to quantify the number of bored berries.

In order to assess the occurrence of natural enemies of the CLM, including predators and parasitoids, all mined leaves found previously were collected from the field and transported to the laboratory for further examination. The focus was on identifying any holes or tear in the mines indicating potential predation. Additionally, the petioles of the mined leaves were carefully placed in small plastic glasses containing water, ensuring the leaves remained turgid. The leaves were placed inside transparent plastic pots of 11 cm height and 7.5 of diameter, covered with a plastic tape. By closely monitoring these pots, any adult CLM or parasitoid wasps that emerged were recorded.

In order to assess the abundance and richness of different arthropod species and guilds (such as herbivores, predators, and pollinators), two sampling methods were employed, tray beating and the use of an entomological net. Tray beating involved gently swinging the coffee branches against a plastic tray (45.5 x 28 x 7.7 cm) containing a thin layer of water, causing arthropods to fall and become trapped. The entomological net was used to capture flying and fast-moving insects that were difficult to collect through tray beating. The net was passed through the air near the coffee plants at a standard distance of 15 m. The collections occurred during the day, between 12:00 and 16:00 after noon.

All captured arthropods were preserved in alcohol and later brought to the laboratory for identification. The specimens were identified at the family level, whenever possible, according to the book “Insects from Brazil” (Rafael et al, 2012). Ants were identified at genus level, whenever possible, according to “Guide to Ant Genus from Brazil” (Baccaro et al, 2016).

2.2. Experiment conducted at Cerrado

The coffee farm is located at Patrocínio, Minas Gerais, Brazil (19°1'21.71"S, 47°2'13.06"W). The species of coffee cultivated is *C. arabica*, cultivar ???, in an agroecosystem and the plants are young, about two years old and had 50 centimeters height. The system consists of coffee with lines with *V. currasavica* e cedar plants. Each *V. currasavica* was spaced by 275 meter from each other in the coffee line. This farm is located at Cerrado biome and have an area of 0.27 hectares. The samples occurred in January, March and April 2023.

In order to evaluate the protective ray distance against the CLM provided by *V. curassavica* in field, we evaluated 20 young coffee plants in fifteen transects of 137.5 meters from a *V. curassavica* plant. We sampled four leaves in two branches from two directions (north and south) (n = 1200), assessing the number of mined leaves by CLM. One plant was evaluated every 12.5 meters, in the standart distances of 0, 12.5, 25, 37.5, 50, 62.5, 75, 87.5, 100, 112.5, 125, 137.5, 150, 162.5, 175, 187.5, 200, 212.5, 225, 237.5, 250, 262.5 and 275 m for CLM active mines.

2.3. Data analysis

For the pest damage, we fitted the data on Generalized Linear Models (GLM) with a negative binomial distribution, considering the count of CLM or CBC infestation as the response variable and the collection date and treatment (conventional and diversified plots) as explanatory variables, as well as their interaction.

For the data on guild and family diversity, a GLM with binomial distribution was applied using the count of specimens from the same family as response and treatment as explanatory. We computed abundance and richness and fitted linear models (with a Gaussian distribution). Abundance or richness was considered as the response variable, while collection date and treatment were treated as explanatory variables, along with their interaction. In these models, if necessary, the response variable was log-transformed to meet the assumption of normality. Also, we compared the abundance of families between diversified and conventional plots of families. Families with less than 10 individuals were not compared.

For the diversity data, interpolation and extrapolation were performed to predict the accumulation curve of families and guilds. Extrapolated richness and diversity indices, such as Shannon and Simpson indices, were calculated. Furthermore, a composition analysis was conducted using the presence-absence data of families and guilds, which were used to construct dissimilarity matrices based on Jaccard distances. The statistical significance between treatments was assessed using permutational multivariate analysis of variance (PERMANOVA) with 1000 permutations. Principal Coordinate Analysis (PCoA) was then applied to visualize the results.

For the evaluation of the ray distance of protection provided by *V. curassavica* against CLM, a Generalized Linear Model (GLM) with a negative binomial distribution was also fitted. The count of mined leaves was considered as the response variable, and distance was the explanatory variable. All data analyses were made with software R (version 4.3.1).

3. RESULTS

3.1. Experiment conducted in a coffee farm at Zona da Mata Mineira

We found no significant differences in the infestation levels between control and diversified plots or either variation of infestation levels along the time for both CLM and CBB (Tables S1 and S2). The mean percentage of mined leaves was 0.12% and 0.13% per plant on diversified and in conventional plots, respectively (Figure 3). The mean percentage of bored berries was 1.5% per plant on diversified and 1.35% conventional plots (Figure 4).

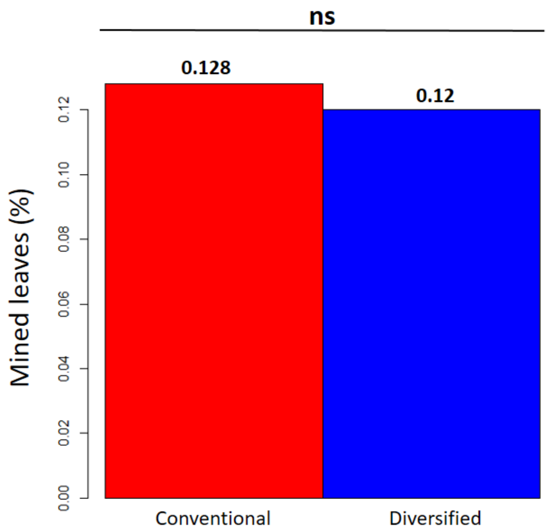

Figure 3. Mean of mined leaves (n = 280, each treatment) on conventional and diversified plots at Zona da Mata Mineira. P-value obtained by GLM with binomial negative distribution.

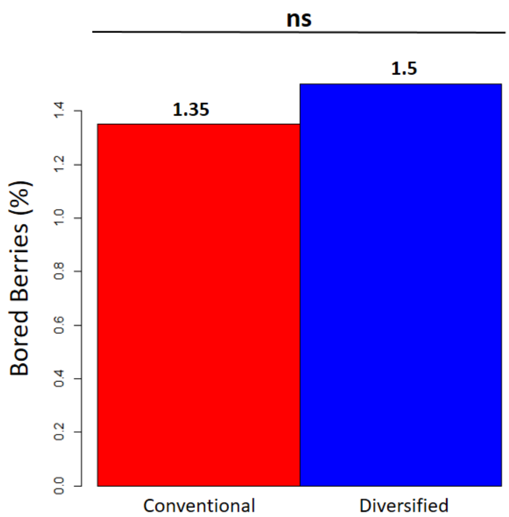

Figure 4. Mean of bored berries (n = 240, each treatment) on conventional and diversified system. P-value obtained by GLM with binomial negative distribution

From 73 mined leaves collected and analyzed during all period of sampling, only one parasitoid emerged from a single mined leaf. The Hymenoptera parasitoid belongs to the Eulophidae family. This tiny rate of parasitism made it impossible to analyze statistically this data.

We have sampled 7262 arthropods on both tray beating and entomological net samplings during the sampling period. These arthropods belongs to 15 orders and 120 families

(Table 1). The most abundant orders were Diptera (48%), Hymenoptera (16%), Araneae (11%), Hemiptera (9%) and Coleoptera (5%) (Table 1).

Table 1. Classification, number of specimens and statistical differences of each taxa within treatments and guilds.

Classification	**Number of specimens**				
Diptera	**Diversified**	**Conventional**	**Total**	**p-value**	**Guild**
Ceratopogonidae	1553	841	2394	0.158	Hematophagous
Chloropidae	264	188	452	0.331	Phytophagous
Ephydridae	127	95	222	0.501	Saprophagous
Chironomidae	93	85	178	0.900	Detritivores
Dolichopodidae	32	31	63	0.947	Predators
Syrphidae	25	27	52	0.861	Predators
Phoridae	15	9	24	0.344	Saprophagous
Drosophilidae	11	9	20	0.714	Frugivorous
Hybotidae	9	10	19	0.839	Predators
Sciaridae	13	5	18	0.063	Saprophagous
Lauxaniidae	12	5	17	0.180	Phytophagous
Tachiinidae	12	3	15	0.049*	Parasitoids
Muscidae	4	9	13	0.207	Saprophagous
Tephritidae	7	6	13	0.790	Frugivorous
Culicidae	9	1	10	0.204	Hematophagous
Faniidae	3	7	10	0.246	Saprophagous
Limoniidae	4	4	8	I	Phytophagous
Sphaoceridae	7	0	7	I	Saprophagous
Cecidomyidae	3	3	6	I	Phytophagous
Platypezidae	2	3	5	I	Mycophagous
Mycetophilidae	3	1	4	I	Mycophagous

Clusiidae	0	2	2	I	Unknown
Ulidiidae	2	0	2	I	Phytophagous
Stratiomyidae	0	1	1	I	Detritivores
Total	**2210**	**1345**	**3555**		
Hymenoptera					
Formicidae	399	557	956	-	
Brachymyrmex	163	324	487	0.042*	Generalists
Crematogaster	125	129	254	0.962	Generalists
Camponotus	51	48	99	0.880	Generalists
Solenopsis	27	28	55	0.949	Generalists
Pseudomyrmex	15	22	37	0.337	Predators
Dorychoderus	12	0	12	<0.001*	Generalists
Nylanderia	5	3	8	I	Omnivorous
Dorymyrmex	1	2	3	I	Omnivorous
Aphaenogaster	0	1	1	I	Omnivorous
Braconidae	21	22	43	0.891	Omnivorous
Encyrtidae	18	17	35	0.891	Parasitoids
Eulophidae	15	7	22	0.117	Parasitoids
Platygastridae	10	6	16	0.335	Parasitoids
Figitidae	7	8	15	0.838	Parasitoids
Ichneumonidae	8	6	14	0.666	Parasitoids
Chalcididae	11	1	12	0.006*	Parasitoids
Scelionidae	8	3	11	0.316	Parasitoids
Ceraphronidae	5	3	9	I	Parasitoids
Diapriidae	3	5	8	I	Parasitoids
Crabronidae	3	3	6	I	Predators
Mymaridae	2	4	6	I	Parasitoids

Eurytomidae	1	4	5	I	Parasitoids
Evaniidae	3	2	5	I	Parasitoids
Aphelinidae	2	1	3	I	Parasitoids
Apidae	1	2	3	I	Pollinators
Bethylidae	1	1	2	I	Parasitoids
Cynipidae	1	0	1	I	Phytophagous
Pteromalidae	0	2	2	I	Parasitoids
Vespidae	0	2	2	I	Predators
Eupelmidae	0	1	1	I	Parasitoids
Halictidae	1	0	1	I	Pollinators
Trichogrammatidae	1	0	1	I	Parasitoids
Total	**522**	**658**	**1180**		
Araneae					
Theridiidae	141	112	253	0.474	Predators
Salticidae	98	101	199	0.850	Predators
Scytodidae	54	45	99	0.581	Predators
Araneidae	45	43	88	0.907	Predators
Cheirachantidae	44	24	68	0.067	Predators
Linyphiidae	41	10	51	0.002*	Predators
Oxyopidae	6	9	15	0.520	Predators
Thomiisidae	7	3	10	0.2427	Predators
Tetragnathidae	2	7	9	0.156	Predators
Lycosidae	7	1	8	I	
Total	**445**	**355**	**800**		
Hemiptera					
Cicadellidae	172	170	342	0.955	Phytophagous
Pentatomidae	35	25	60	0.321	Phytophagous

Geocoridae	30	16	47	0.037*	Predators
Miridae	17	23	40	0.530	Phytophagous
Aphididae	9	28	37	0.166	Phytophagous
Reduviidae	17	11	28	0.283	Predators
Coreidae	10	16	26	0.444	Phytophagous
Scutelleridae	8	15	23	0.298	Phytophagous
Delphacidae	10	9	19	0.834	Phytophagous
Lygaeidae	3	4	7	I	Phytophagous
Rhopalidae	2	4	6	I	Phytophagous
Membracidae	5	1	6	I	Phytophagous
Nabidae	0	5	5	I	Predators
Cixiidae	1	2	3	I	Phytophagous
Psylloidea	2	1	3	I	Phytophagous
Derbidae	1	1	2	I	Phytophagous
Flatidae	1	1	2	I	Phytophagous
Pyrhhocoridae	1	1	2	I	Phytophagous
Cydnidae	1	0	1	I	Phytophagous
Tropiduchidae	0	1	1	I	Phytophagous
Total	**325**	**335**	**660**		
Coleoptera					
Chrysomelidae	85	85	170	1	Phytophagous
Coccinellidae	15	29	44	0.082	Predators
Mordellidae	17	13	30	0.644	Phytophagous
Staphylinidae	15	14	29	0.888	Predators
Curculionidae	10	9	19	0.818	Phytophagous
Phalacridae	4	15	19	0.012*	Saprophagous
Meloidae	8	8	16	1	Parasitoids

Tenebrionidae	8	6	14	0.592	Detritivores
Anthicidae	5	7	12	0.566	Generalists
Latrididae	6	6	12	1	Saprophagous
Nitidulidae	7	5	12	0.562	Saprophagous
Carabidae	7	3	10	0.246	Predators
Cryptophagidae	5	2	7	I	Mycophagous
Cantharidae	0	6	6	I	Predators
Lampyridae	5	1	6	I	Predators
Mycetophagidae	4	1	5	I	Mycophagous
Scarabeidae	2	1	3	I	Phytophagous
Cucujidae	0	2	2	I	Generalists
Anobiidae	1	0	1	I	Unknown
Byrhidae	1	0	1	I	Detritivores
Cerambycidae	1	0	1	I	Xylophagous
Clambiidae	0	1	1	I	Mycophagous
Elateridae	1	0	1	I	Phytophagous
Lycidae	1	0	1	I	Phytophagous
Total	**208**	**214**	**422**		
Blattodea					
Blattellidae	151	145	296	0.853	Detritivores
Psocodea					
Psocidae	21	33	54	0.204	Detritivores
Ectopsocidae	12	3	15	0.016*	Unknown
Total	**33**	**36**	**69**		
Lepidoptera					
Larvae[ni]	19	16	35	0.695	Phytophagous

Lyonetiidae	11	16	27	0.589	Phytophagous
Tineidae	9	7	16	0.759	Phytophagous
Pyraloidea	2	7	9	I	Phytophagous
Total	**41**	**46**	**87**		
Entomobryomorpha (Collembola)[ni]	21	39	60	0.313	Detritivores
Orthoptera					
Tettigoniidae	7	14	21	0.183	Phytophagous
Romaleidae	7	9	16	0.668	Phytophagous
Gryllidae	6	7	13	0.813	Omnivorous
Acridiidae	0	3	3	I	Phytophagous
Total	**20**	**33**	**53**		
Thysanoptera					
Phlaeothripidae	13	**7**	20	0.380	Unknown
Thripidae	5	8	13	0.403	Unknown
Total	**18**	**15**	**33**		
Dermaptera					
Forficulidae	4	8	12	0.493	Ownivorous
Labiduridae	5	4	9	I	Detritivores
Total	**9**	**12**	**21**		
Neuroptera					
Hemerobiidae	8	6	14	0.639	Predators
Chrysopidae	2	4	6	I	Predators
Total	**10**	**10**	**20**		
Mantodea					
Mantoididae	0	1	1	I	Predators

Acanthopidae	4	0	4	I	Predators
Total	**4**	**1**	**5**		
Odonata					
Caenagrionidae	0	1	1	I	Predators

I: Insufficient data for analyses
*: Significant difference ($p < 0.05$)
ni: Not identified until family level

Concerning to the natural enemies, we found predators from 17 insect families (Syrphidae, Dolichopodidae, Hybotidae, Formicidae, Crabronidae, Vespidae, Carabidae, Lampyridae, Coccinellidae, Cantharidae, Geocoridae, Reduviidae, Nabiidae, Hemerobiidae, Chrysopiidae, Achantopidae and Mantoididae) and 10 spider families (Scytodidae, Linyphiidae, Salticidae, Oxyopidae, Thomiisidae, Theridiidae, Lycosidae, Cheirachantidae, Tetragnathidae and Araneidae) (Table 1).

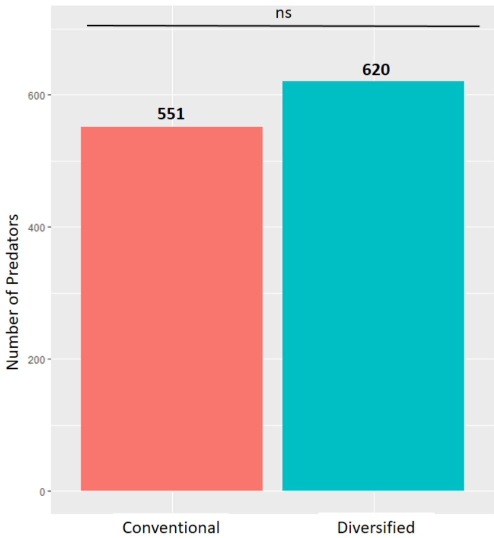

Figure 5. Total number of predators found in conventional and diversified plots at Zona da Mata Mineira with entomological net and tray beating (n = 28, each treatment).

We did not find significant differences in the total number of spiders and predatory insects sampled in both the control and diversified plots ($p = 0.714$) (Figure 5). However, we did observe a significant difference in the abundance of the Geocoridae predatory bugs and spiders of the family Linyphiidae (Table 1).

We identified 19 families of parasitoids, including one from the Diptera order (Tachinidae) and the remaining families belonging to the Hymenoptera (Encyrtidae, Aphelinidae, Braconidae, Ichneumonidae, Chalcididae, Ceraphronidae, Eulophidae, Mymaridae, Trichogrammatidae, Diapriidae, Figitidae, Platygastridae, Eurytomidae, Bethylidae, Scelionidae, Pteromalidae, Evaniidae, and Eupelmidae) (Table 1).

We did not find difference in the total number of parasitoids (p = 0.121), but it is possible to note that diversified plots showed a slightly higher number of parasitoids, perhaps not enough to being a significant difference (Figure 6). However, differences were observed in the abundance of the families Tachinidae and Chalcididae, both being more abundant in diversified plots than conventional ones (Table 1).

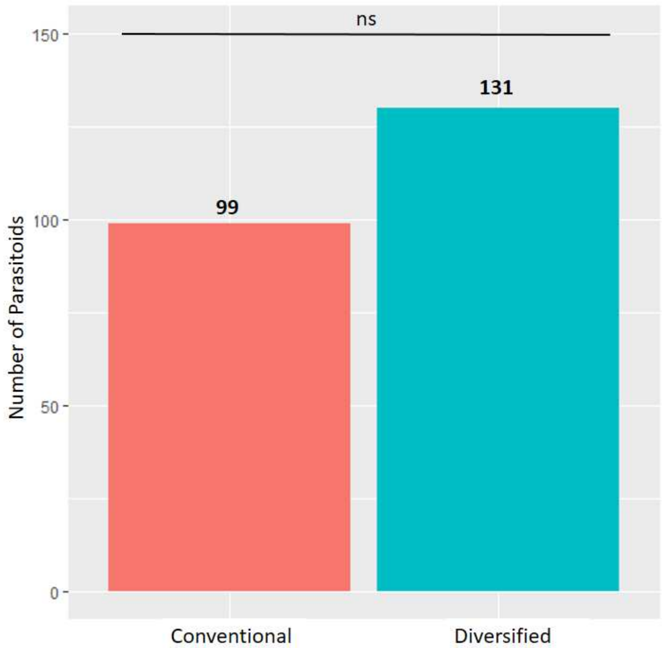

Figure 6. Total number of parasitoids found in conventional and diversified plots at Zona da Mata Mineira with entomological net and tray beating (n = 28, each treatment).

Ectopsocidae family (insects from order Psocodea) and ant genus *Dorychoderus* were also more abundant in diversified than conventional plots. *Brachymyrmex* ants and *Phalacridae* bettles were more abundant in conventional plots than diversified ones.

We observed an increase from January to May on both abundance (p = 0.018) and richness (p < 0.001) of insect families (Figure 7). Abundance of families did not vary between treatments, but the diversified plots had a more pronounced increase in richness of families over time (p = 0.049) (Figure 7).

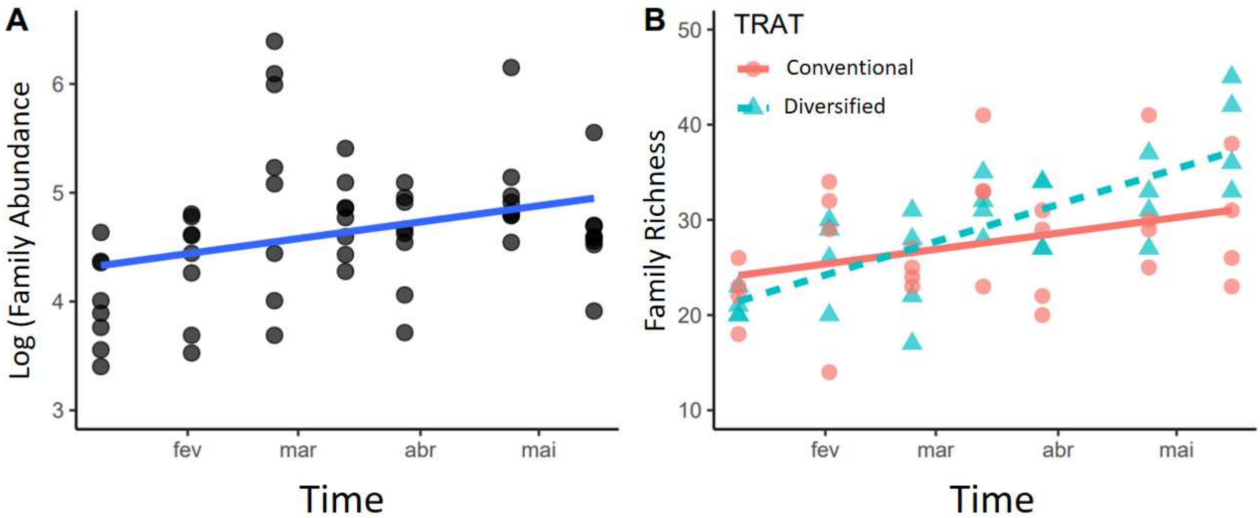

Figure 7. Abundance (A) and richness (B) of families on conventional and diversified plots (n = 56)

Abundance and richness of guilds has increased with time (p = 0.018 and 0.002, respectively) but did not vary between the treatments (Figure 8).

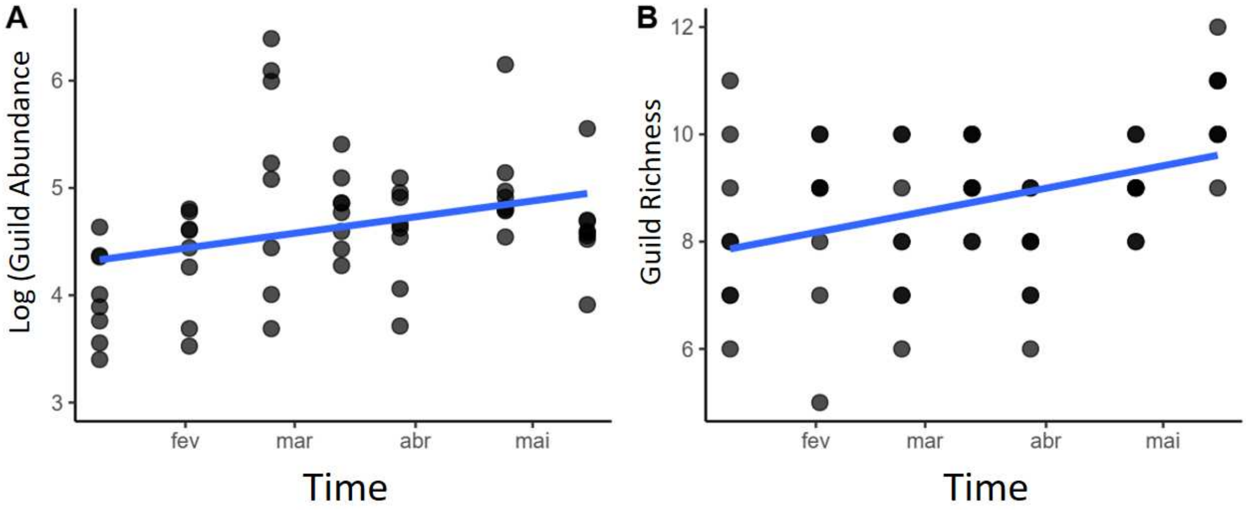

Figure 8. Abundance (A) and richness (B) of guilds on conventional and diversified plots (n = 56)

Analyzing the accumulation curve of families and guilds (Figure 9) and diversity index (Figure 10), we observed no differences between family diversity in conventional and diversified plots. Guild diversity, on the other hand, was higher on diversified plots and is predicted to increase with the presence of *V. curassavica.* No differences on diversity index of Shannon and Simpson were observed between treatments for both families and guilds.

Principal coordinators analysis (PCoA) also evidenced no significant differences on family and guild diversity between treatments. It is possible to see that ecological structure for both conventional and diversified plots were almost the same during sample period (Figure 11).

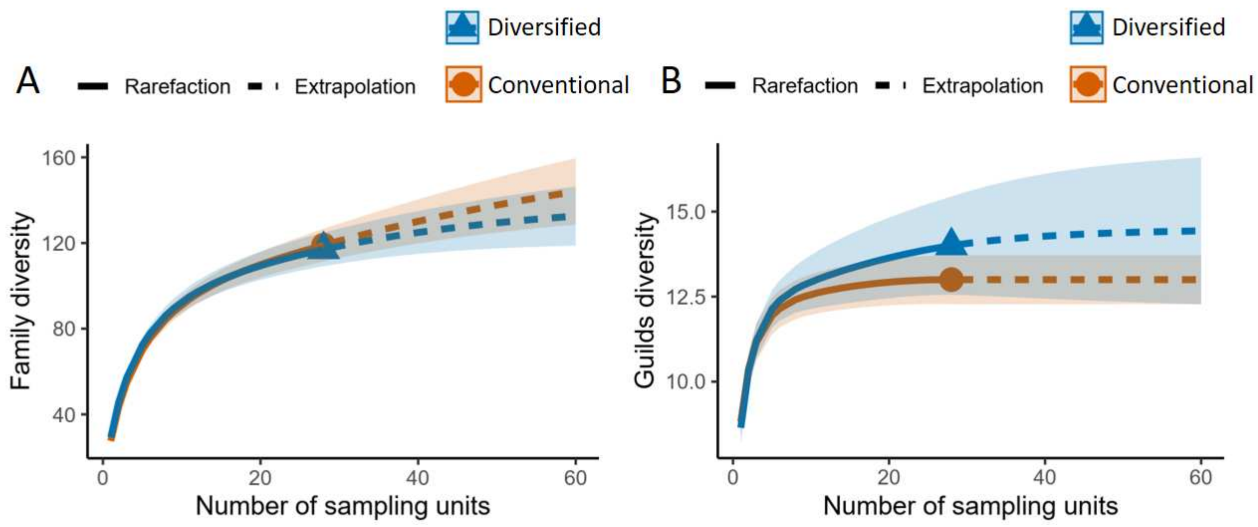

Figure 9. Accumulation curve of (A) families and (B) guilds (95% of confidence interval) on relation to sampling units (n = 28, each treatment) and predicted extrapolation in diversified and conventional plots.

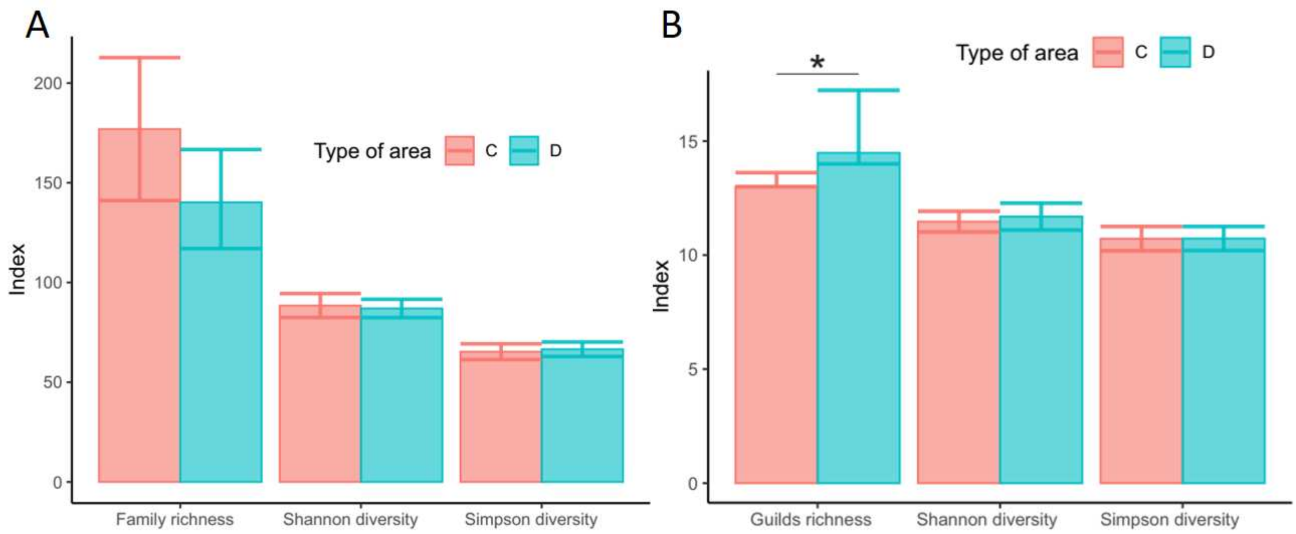

Figure 10. Diversity index of families (A) and guilds (B). Bars indicate the predicted values by extrapolation of accumulations curves with confidence interval (n = 28, each treatment). * Indicate significant differences at 95% of confidence. The C are conventional plots and D are diversified plots.

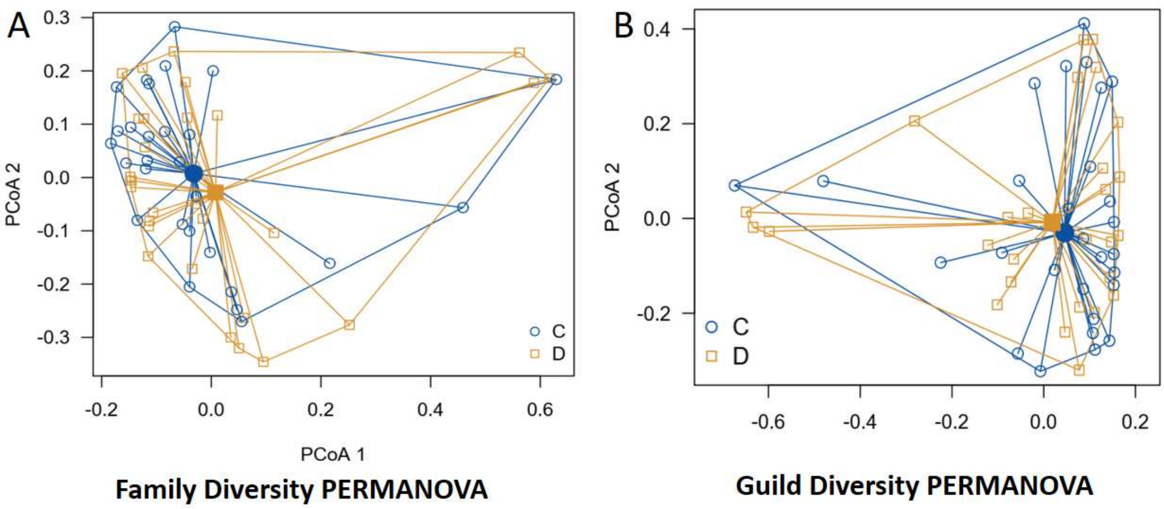

Figure 11. Principal coordinates analysis (PCoA) ordination from Jaccard dissimilarity matrix between treatments based on (A) family and (B) guild diversity. Treatment differences were compared by permutational multivariate analysis of variance (PERMANOVA). Each point represents a sample (n= 28, each treatment), and the segments join points to their centroid.

3.2. Experiment conducted at Cerrado

We observed no changes on the level of infestation of CLM with increasing of distance (from 0 to 137.5 m) (p = 0.465, see table S3). The mean infestation rate in the coffee farm in Cerrado with agroecosystem was 0.18% of mined leaves per plant.

4. DISCUSSION

The lack of significant difference on infestation levels of CBC and CLM between conventional and diversified plots in the Zona da Mata might have occurred due to the conventional system used in the farm. Even in the presence of natural enemies (Table 1), the low pest infestation rate can be mainly attributed to chemical and augmentative biological control. With the application of pesticides or biological agents, such as entomopathogenic fungi, the populations of both coffee berry borer and coffee leafminer were kept low. For the

CBB, even below the economic injury level (Fernandes et al, 2011).throughout the sampling period.

It is reasonable to assume that, even with the attraction and resources provided by a companion crop, some natural enemies have their populations reduced or absent in a conventional coffee system (Stark, Vargas and Banks, 2007). Therefore, to better assess the impact of *V. curassavica* on CBB and CLM damage in coffee farms, a system with reduced or no use of chemical inputs should provide stronger evidence of its effectiveness.

Concerning to CLM natural enemies, the tiny parasitoid rate likely occurred due to two reasons. First, the infestation rate of CLM was already low (Figure 4). Second, there is a possible sensitivity of CLM parasitoids to chemical inputs, particularly pesticides used in the conventional system. It is well known that some pesticides can have negative effects not only on target organisms such as the CBB and CLM, but also on pollinators, predators, parasitoids, and other species within the environment (Pereira et al., 2009; Pisa et al., 2015; El-Wakeil et al., 2013). Another reason for the absence of parasitoids from CLM is the limited availability of plant-based resources in a monoculture landscape, what can decrease their survival rate (Wan et al, 2019). The parasitoid wasps rely on their hosts solely during the larval stage, while adult wasps primarily depend on sugar and pollen for survival and to increase their lifespan (Tylianakis, Didham and Wratten, 2004; Calderón-Arroy et al, 2023). As coffee plants do not consistently provide these resources along the year, populations of parasitoid wasps may suffer from a lack of resources, leading to a significant reduction or even absence of these parasitoids.

In this scenario, the addition of a non-crop plant like *V. curassavica*, which can provide resources to CLM parasitoids (Calderón-Arroy et al, 2023), would benefit their populations. Possibly, the density and plant size and short time of evaluation may not have be sufficient to overcome all negative factors in the conventional system that limit the role of parasitoids in controlling the CLM population. To better assess the potential of biological control by these natural enemies on coffee farms, a reduction in the use of insecticides must be considered. This reduction would enable the populations of these organisms to survive in this agricultural environment.

Furthermore, the samplings occurred during the rainy season in Minas Gerais, Brazil, when the populations of both pests are naturally lower due the impact of rainfall on mortality of these insects (Pereira et al, 2007; Rodríguez et al, 2013), what probably contributed to the low infestation observed in this study in all plots. An extent of the sampling period may show deeper differences between conventional and diversified plots since that dry season (which in Brazil in the second semester of the year) contribute to a significant higher level of the infestation of both CBB and CLM (Pereira et al, 2007; Rodríguez et al, 2013).

The potted *V. curassavica* used in the field also had some variations in height and development stage. Some plants have rooted in the ground and developed in height, produced flowers and fruits and were healthy. Unfortunately, other plants did not rooted, developed less or did not developed and did not produced neither flowers or fruits. This mismatch on plant development can be attributed to the use of plant pots, which made it difficult to the plants asses nutrients and water from soil. With this in mind, the potted *V. curassavica* may did not expressed their full potential in attracting the natural enemies and suppress the infestation of the CBC and CLM.

Geocoridae bugs were approximately twice as abundant in the diversified plots compared to the conventional ones (Table 1). These heteropterans are well-known as generalist predators found in various agricultural ecosystems worldwide (Kobor, 2020). They feed on a range of agricultural pests, including aphids, thrips, and caterpillars, although they may also consume plant parts such as seeds and pollen when prey is scarce or absent (Stoner, 1970). *Varronia curassavica* can provide both pollen and seeds, enhancing the survival of Geocoridae bugs since this shrub produces flowers and seeds over the year (Brandão et al., 2015; Martins, 2017).

Linyphiidae are the second most diverse family of spiders, after only by Salticidae (Sharma, Singh and Singh, 2020). Spiders are predators that have a very important hole in ecosystems worldwide, especially in controlling insect populations. Besides their ubiquitous presence on agroecossystems, spiders are less studied than insects, but can play an important hole as biologic agent control (Riechert and Lockley, 1984). As an example, the spider *Pardosa pseudoannulata* is considered a successful biological agent control on rice fields, helping to suppress population of the main rice pests (Riechert and Lockley, 1984). In general, spiders are polyphagous predators, feeding on more than one prey, what turns even challenger inferring about its importance in coffee farms without a further identification and deep look research (Riechert and Lockley, 1984; Hodge, 1999; Sigsgaard, Toft and Villareal, 2001).

Tachinidae is a diverse and ecologically important family of parasitoid flies, being important natural enemies in many terrestrial ecosystems, including agricultural landscapes. They parasite a wide range of insects and other arthropods, especially Lepidoptera larvae and other herbivores insects (Grenier, 1988; Stireman, O'Hara and Wood, 2006).

Chalcididae is a family of parasitoid wasp with over than 1500 species, being more diversified on tropical areas (Delvare, 1992). Wasps from this family can parasite many holometabolous insects from many orders (Lotfalizadeh, Ebrahimi and Delvare, 2012). Some species are used as effective agents of biological control in South America against defoliators insects beetles and caterpillars and in North America against the gypsy moth (*Lymantria dispar*) (Pereira et al, 2013; Roscoe, 2014; Delvare, 2017).

The fact that diversified plots improve the abundance of both Tachinidae flies and Chalcididae wasps support the use of *V. curassavica* as an efficient companion plant in order to improve the conservation biological control, attracting these natural enemies and supporting their lifespan, leading to the enhancement of natural pest control in coffee and other crops.

Ectopsocidae and *Dorychoderus* ants were also more abundant in diversified than conventional plots. The Ectopsocidae family is understudied and not many information is available about this taxa in Brazil (Silva-Neto, Andrade and Aldrete, 2013, Rafael et al, 2012). Besides the difference in *Dorychoderus*, it is not possible to attribute these differences to treatments since all 12 specimens were sampled once and in only one sample from 56 samples, which means that the data is insufficient.

Brachymyrmex ants and *Phalacridae* beetles, in the other hand, were more abundant in conventional plots. These differences may not be directly correlated to presence or absence of *V. curassavica*, *Brachymyrmex* ant distribution depends more on the availability of suitable soils, rocks or logs to nidify (Quirán, 2004; Baccaro et al, 2016). This mean that eventual abiotic differences between the plots may explain this difference. Phalacridae beetles are mycophagous insects, associated directly to the presence of fungi (Rafael et al, 2012). The larvae habit are unknown for Brazilian species. The reason of the difference between treatments remains uncertain.

Family diversity results shows that the richness of families in diversified plots increased more with time than conventional ones. This means that the use of *V. curassavica* as companion plant on the coffee farms led to an increase in biodiversity in the coffee farm. Although guilds did not vary between treatments, the extrapolation curves indicate a tendency to guild richness to increase and possibly to overcome the conventional system richness. These results evidence the fact that crop diversification lead to increase biodiversity and ecological health in agroecosystems, promoting a conservation and sustainable environment as well as increase the number of beneficial organisms.

Several studies show the association of the increase in biodiversity and the increase of natural pest control. Bianchi et al (2006) revised the available data about the relationship between biodiversity and natural enemies and found that in 74% of studied papers the increase in the complex of landscapes translated in an increase of natural enemies and ecosystems services. Tschumi et al (2016) studied the effect of the sown flower strips and verified that this plant can increase the number of hoverflies and lacewings by 127% and 48%, respectively, on potato crops and the reduction of aphids by 75%. Wan et al (2019) showed that plant diversification on peach orchards leads to an increase of 38.1% on natural enemies and decrease of 16.9% on herbivore insects.

It is also important to note that not only the number of beneficial organisms has increased, but the phytophagous and the key pest populations kept the same and did not raised too. Verifying these data on field is essential to promote scientific based strategies of sustainable food production with security and efficiency.

The evaluation of the distance ray of protection provided by *V. curassavica* against CLM showed a low infestation rate, with no difference among distance, that can be explained once more, by the rainy season, when the infestation levels are naturally low. Furthermore, Nascentes et al (2021) studied the infestation of CLM and observed that the months with a higher incidence of this pest was April to August in the Cerrado.

The results may suggest a protection of coffee plants by *V. curassavica* against *L. cofeella*, since the mean infestation rate was low across all studied transect. However, it is not possible to conclude if the infestation levels were low mainly by this protection or by the abiotic circumstances and seasonal changes. A study on this protective effect must be continued during the rainy and dry seasons to confirm or reject these results.

5. CONCLUSIONS

We found no differences in the infestation levels of both coffee berry borer (*Hypothenemus hampeii*) and coffee leaf miner (*Leucoptera coffeella*) between conventional and diversified plots, probably due to the chemical control and limited sampling period (January to May).

Only one parasitoid of *L. coffeella*, belonging to Eulophidae family was found on all samples, indicating a tiny occurrence of these natural enemies in the conventional system at Zona da Mata Mineira.

The number of predators and parasitoids was slightly higher on diversified than conventional plots, but not sufficient to fit statistical difference. However, the predatory bug of family Geocoridae, spiders of Linyphiidae family, the parasitoid flies from Tachinidae family and the parasitoid wasps of Chalcididae family were more abundant on diversified plots, indicating that *V. curassavica* is a suitable plant to attract these natural enemies

We found that richness of families increased with time on diversified plots and the guild richness tended to increase with time in extrapolation curves, showing that *V. curassavica* can improve the biodiversity, increasing the ecosystems services provided by beneficial organisms.

Finally, the mean infestation rate of *L. coffeella* on the diversified system in Cerrado was low and no differences were observed on the infestation levels across the transect from 0 to 137.5 meters away from *V. curassavica*. More studies carried out during pest favorable season must confirm if *V. curassavica* promoted this suppression on *L. coffella* population

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SUPPLEMENTARY MATERIAL

Table S1 – Results of Anova (Binomial Negative) of response variable "bored berries" and independent variables TRAT (Conventional and Diversified) and DATE (Time).

	DF	DEVIANCE	RESID. DF	RESID. DEV	PR (>CHI)
NULL			479	506.69	
TRAT	1	0.56076	478	506.13	0.4540
DATE	1	0.68675	477	505.44	0.4073
TRAT:DATE	1	1.59604	476	503.84	0.2065

Table S2 – Results of Anova (Binomial Negative) of response variable "mined leaves" and independent variables TRAT (Conventional and Diversified) and DATE (Time).

	DF	DEVIANCE	RESID. DF	RESID. DEV	PR (>CHI)
NULL			559	243.28	
TRAT	1	0.04788	558	243.28	0.8268
DATE	1	1.41411	557	241.82	0.2344
TRAT:DATE	1	0.45319	556	241.37	0.5008

Table S3 – Results of Anova (Binomial Negative) of response variable "mined leaves" and independent variable Distance.

	DF	DEVIANCE	RESID. DF	RESID. DEV	PR (>CHI)
NULL			314	182.23	
TRAT	1	0.53335	313	181.70	0.4652