



**VITOR BARRILE TOMAZELLA**

**IMPLICATIONS OF DIVERSIFICATION IN COFFEE  
CROP ON FOUR PARASITOID FAMILIES**

**LAVRAS - MG  
2020**

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Thesis presented to Universidade Federal de Lavras as part of the exigency of the Post-Graduate program of Entomology, Biological Control concentration area, to obtention of PhD title.

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PARASITOID FAMILIES  
IMPLICAÇÕES DA DIVERSIFICAÇÃO EM CULTIVOS DE CAFÉ SOBRE  
QUATRO FAMILIAS DE PARASITOIDES**

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## DEDICATORY

*Aos meus Pais,  
À minha esposa,  
Aos meus filhos.  
Dedico*

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## ABSTRACT

The simplification of the agriculture using mainly monoculture imply in huge loss to diversity and other ecological services, such as pest control. Coffee crop, in Brazil, is typically produced under this system of production with large areas of monoculture. But, in southern Minas Gerais, even the production being monocultural, the local landscape is mainly a mosaic of vegetation fragments and, these vegetation improve the diversity of parasitoids, a regional aspect that helps the improvement of the diversification. Another way of diversification is the utilization of shade trees intercropped with the coffee, that also have lot of benefits, such as increase in the parasitoids diversity. By general, many works showed these increments to all families of parasitoids, but only few works were conducted aiming the beneficial families only. Our work goal was to figure out what is the contribution of native vegetation, diversified coffee and shade in coffee in the diversity, abundance and richness of four parasitoid families that have many important coffee pests parasitoids. This work was carried in two farms on southern of Minas Gerais, Brazil, on with coffee crop intercropped with different tree species, being them, Avocado, Mangium, Macadamia, Cedar and Teak and a native surrounding vegetation, and another with a shaded coffee and a full sun coffee. We collect parasitoids at the diversified coffee, the one intercropped, at native vegetation and a monocultural coffee crop. At the second, we collected parasitoids in the shaded coffee and full sun coffee. In both cases, the collects were carried from 2016 to 2018, every three months, utilizing yellow pan traps that stayed at the field for a period of 48 hours. The insects were taken to the Laboratório de Controle Biológico Conservativo of Universidade Federal de Lavras to be identified at morphospecies. At the first farm, we analysed two things, the first, was the influence of the trees used to diversify the coffee crop on the four families of parasitoids and if there were difference in these diversity from a monoculture of coffee; the second was to find differences between the intercropped coffee, the monoculture coffee and the native vegetation on the same four parasitoids families. On the second farm, we analysed the effect of shaded coffee and full sun coffee on the community of the same four parasitoid families. Our results, for the first farm and the first case, were that the Avocado (0.19) and Full Sun (0.07) coffee showed similar results for diversity and both lower than the founds for the other plants: Mangium (0.57), Cedar (0.42), Macadamia (0.38) and Teak (0.32). For the second case at the first farm we found a difference in all three treatments, being the Diversified (1.64), Native (1.09) and Monoculture (0.07) all different with the diversified being the greater. For the second farm, we found a well visible difference from shaded (0.59) and full sun (0.32) diversity. We this, we concluded that, the diversification, improve the diversity of the parasitoid families and also, the shade at coffee plantation brings benefits to it.

**KEYWORDS:** Braconidae. Eulophidae. Bethylidae. Mymaridae. Conservation Biological Control. Shading Trees.

## RESUMO

A simplificação da agricultura usando principalmente a monocultura implica em enormes perdas para a diversidade e outros serviços ecológicos, como o controle de pragas. A cultura do café, no Brasil, é tipicamente produzida sob esse sistema de produção com grandes áreas de monocultura. Porém, felizmente, no sul de Minas Gerais, mesmo sendo a produção em sua maioria em monocultura, a paisagem local é um mosaico de fragmentos de vegetação e, esses corredores e fragmentos de vegetação melhoram a diversidade de parasitóides, aspecto regional que ajuda na melhoria da diversificação. Outra forma de diversificação é a utilização de árvores consorciadas com o café, que também trazem muitos benefícios, como aumento da diversidade de parasitóides e outros animais. De maneira geral, muitos trabalhos mostraram esses incrementos para todas as famílias de parasitóides, mas apenas alguns foram realizados visando apenas as famílias benéficas. Nosso objetivo de trabalho foi descobrir qual é a contribuição da vegetação nativa, café diversificado e sombra no café na diversidade, abundância e riqueza de quatro famílias de parasitóides que possuem muitos importantes parasitóides de pragas do café. Este trabalho foi realizado em duas fazendas no sul de Minas Gerais, Brasil, com cultivo de café consorciado com diferentes espécies de árvores, sendo elas: Abacate, Mangium, Macadâmia, Cedro e Teca e uma vegetação nativa do entorno, e outra com café sombreado e um café cheio de sol. No primeiro momento, coletamos parasitóides no café diversificado, na vegetação nativa e em uma lavoura de café em monocultura. No segundo, coletamos parasitóides no café sombreado e no café a pleno sol. Nos dois casos, as coletas foram realizadas de 2016 a 2018, a cada três meses, utilizando armadilhas amarelas que permaneceram no campo por um período de 48 horas. Os insetos foram levados ao Laboratório de Controle Biológico Conservador da Universidade Federal de Lavras para serem identificados nas morfoespécies. Na primeira fazenda, analisamos duas coisas, a primeira, foi a influência das árvores usadas para diversificar a cultura do café nas quatro famílias de parasitóides e se havia diferença nessa diversidade de uma monocultura de café; o segundo foi encontrar diferenças entre o café diversificado, o café monocultivo e a vegetação nativa nas mesmas quatro famílias de parasitóides. Na segunda fazenda, analisamos o efeito do café sombreado e a pleno sol na comunidade das mesmas quatro famílias de parasitóides. Nossos resultados, para a primeira fazenda e o primeiro caso, foram que o café Abacate (0,19) e Full Sun (0,07) apresentaram resultados semelhantes para a diversidade e ambos inferiores aos encontrados para as outras plantas: Acácia (0,57), Cedro (0,42), Macadâmia (0,38) e Teca (0,32). Para o segundo caso na primeira fazenda, encontramos uma diferença, diversidade, nos três tratamentos, sendo o Diversificado (1,64), Nativo (1,09) e Monocultura (0,07), todos diferentes, sendo o diversificado o maior. Para a segunda fazenda, encontramos uma diferença bem visível da diversidade no café sombreado (0,59) e pleno sol (0,32). Concluimos que, a diversificação, melhora a diversidade das famílias parasitóides estudados e que o sombreamento também apresenta aspecto favorável.

**PALAVRAS-CHAVE:** Braconidae. Eulophidae. Bethyidae. Mymaridae. Controle Biológico Conservativo. Árvores para Sombreamento.



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## **FIRST PART**

## 1 GENERAL INTRODUCTION

Coffee is a tropical perennial shrub plant from *Coffea* L. genus with two species of economic importance, *C. arabica* (Gentianales: Rubiaceae) (Linnaeus, 1753). and *C. canephora* (Gentianales: Rubiaceae) (Pierre ex Froehn, 1987), being that *C. arabica* historically comprehend around 70% off the world and Brazil production. It is adapted to humid climates with mild temperatures and can remain in the field for more than 25 years.

Being a perennial plant, means that it is subjected to environmental factors and the surrounding landscape influence for a long period, exchanging many animals and ecological services with the compounds of the landscape it is inserted. As expected, its productivity is closely related to rain regime, where it is not irrigated, and other several biotic factors as insects, which can cause enormous damage to the crop and significantly drop the production.

It is known that the simplification of the habitat contributes significantly to the decrease of some important ecological services, as pest regulation and pollination and the loss of important habitats and, coffee plantation in Brazil is mainly produced under conventional plantation system where there was great fragmentation of the habitat to give space to monocultures, resulting in its simplification and so, being a more suitable habitat to pests like coffee borer beetle (*Hypothenemus hampei*) (Coleoptera: Curculionidae, Scolytinae) (Ferrari, 1867) and coffee leaf miner (*Leucoptera coffeella*) (Lepidoptera: Lyonetiidae) (Guérin-Mèneville, 1842), two main pests of the crop that have the potential to cause enormous damage to the crop in Brazil.

Fortunately, the population dynamics of these pests varies according to the cultivation regions, due to biotic such as natural enemies and abiotic factors that act in the coffee agroecosystem. Some parasitoids as *Phymastichus coffea* (Hymenoptera: Eulophidae) (LaSalle, 1990), *Heterospilus coffeicola* (Hymenoptera: Braconidae) (Schimideknecht, 1924), *Orgilus niger* (Hymenoptera: Braconidae) (Penteado-Dias, 1999), *Stiropius letifer* (Hymenoptera: Braconidae) (Mann, 1872), *Eubazus punctatus* (Hymenoptera: Braconidae) (Ratzeburg, 1852), *Closterocerus coffeellae* (Hymenoptera: Eulophidae) (Ihering, 1914), *Proacrias coffeae* (Hymenoptera: Eulophidae) (Ihering, 1914), *Horismenus aeneicollis* (Hymenoptera: Eulophidae) (Ashmead, 1904), *Prorops nasuta* (Hymenoptera: Bethyridae) (Waterson, 1923) and *Cephalonomia stephanoderis* (Hymenoptera: Bethyridae) (Betren, 1961) are identified as important parasitoids of the leaf miner or borer beetle.

Also, reports in work conducted in Ivory Coast, pointed *Polynema* sp. (Hymenoptera: Mymaridae), *Tineobius* sp. (Hymenoptera: Eupelmidae), *Chelonus* sp. (Hymenoptera: Braconidae), *Bracon* sp. (Hymenoptera: Braconidae) and *Stenobracon* sp. (Hymenoptera: Braconidae) as potential agents to control coffee borer. Mymaridae are very important egg parasitoids of Auchenorrhynca insects that have a potential to cause great damage to the crop.

With the increase in the environment's diversity, natural enemies are helped in several ways, mainly: by offering alternative food for adults, such as nectar, pollen and sugary substances; availability of shelter and adequate microclimate and the presence of prey and alternative hosts for natural enemies. A more diversified coffee culture promotes an increase in the diversity of insects, birds and other organisms that help in the control of possible pests and with other important ecological services, as pollination and pest control.

Conservation biological control is based on maintaining natural enemies in the cultivation area using attractive plants, making a complex spatial arrangement, and maintaining native border vegetation to enhance the ecological service of pest control. Studies point that nearly 65% of the potential damage caused by insects are suppressed by biological control in all aspects, as pest controlled and potential pest that never reach pests status due to natural control, meaning

One important aspect of this technic is identify structures of the landscape that can improve those services, such as Valos in southern Minas Gerais. Valos are corridors of vegetation that originated from the firsts division of the land by its owners and are now unique vegetation structures with cultural and historical importance that act as key landscape structures in relation with ecological services, especially those provided by insects.

In perennial crop systems, this diversification can be done using, for example, three plants. These provide shade, input of organic matter, nutrients, conserve the soil and can represent an extra source of tree resources for rural family activity and can contribute economically by the direct or indirect commercialization of the products of the trees.

The selection of the tree for diversification and shade have to be very cautious, taking in account many aspects, some simple as size, canopy cover, leaf drop, and some more complex as, organic matter and competition, but as positive aspect it can contribute to an increase in the income, with direct utilization of that plant and as well, it allows the product to be commercialized out of the commodity, reaching more attractive prices.

There are not many works conducted in Brazil regarding diversification inside the crop contrasted with diversification outside the crop. And, especially evaluating Valos.

At this work, we had the following goals:

- 1) Determine if the diversification inside coffee crop has the same effect of the native surrounding vegetation and is, both contribute more to diversity, richness, and abundance of parasitoids of the families Braconidae, Eulophidae, Bethylidae and Mymaridae.
- 2) Find out if the shaded coffee has huge differences in the same parasitoid families comparing with full sun coffee
- 3) By last, expecting differences in both above, we aimed to see the individual contribution of some trees intercropped with the coffee crop.

## **2 THEORETICAL REFERENCE**

### **2.1 Coffee crop**

Coffee is a perennial plant from *Coffea* L. genus, having more than hundred species worldwide. However, when we take in account the world production and agronomic importance, only two species take the stage, *C. arabica* L. and *C. canephora* Pierre, being that *C. arabica* historically comprehend around 70% off the world production according to Matiello et al., (2010) and also in Brazil, the greatest world producer, it contributes to 72% (CONAB, 2020; MARTINS, 2008).

It is a shrub tropical altitude plant, adapted to humid climates with mild temperatures, and can remain in the field for more than 25 years. It originated in Ethiopia and came to Brazil in the early 18th century through Sergeant Major Francisco de Mello Palheta, initially arriving in the state that today is Para. Initially a household product, it began to be exported in the late 18th century, (MARTINS, 2008).

Brazil is current largest coffee producer and exporter, with estimated production of 60 million of processed bags in an area of 1885.5 thousands hectares in 2020, being Minas Gerais state the great Brazil's producer with an estimated production of 30 million processed bags. (CONAB, 2020). Over the past three decades, coffee has achieved an average annual production of 24.3 million 60 kg bags of processed coffee, generating millions of direct and indirect jobs. About 70% of coffee growers are classified as small producers, with a maximum

of 20 hectares of coffee area, responsible for 70-80% of the total gross income of these properties (MATIELLO *et al.*, 2010).

Coffee productivity is closely related to rain regime, where it is not irrigated, and other several biotic factors as insects, which can cause enormous damage to the crop and drop significantly the production (REIS; SOUZA; VENZON, 2002). It is known that the simplification of the habitat contributes significantly to the decrease of some important ecological services, as pest regulation, and the loss of important habitats (TSCHARNTKE *et al.*, 2005) and, coffee plantation in Brazil is mainly produced under conventional plantation system where there was great fragmentation of the habitat to give space to monocultures, resulting in its simplification and so, being a more suitable habitat to pests (ALTIERI; SILVA; NICHOLLS, 2003; DIAS *et al.*, 2008).

## 2.2 Main coffee pests and its parasitoids

As it is more suitable, some pests, like coffee borer beetle (*Hypothenemus hampei*) (Ferrari, 1867) (Coleoptera: Curculionidae, Scolytinae) and coffee leaf miner (*Leucoptear coffeella*) (Guérin-Mèneville, 1842) (Lepidoptera: Lyonetiidae), two main pests of the crop, can increase rapidly in numbers and harm the crop, leading to great losses in production (GALLO, 2002; REIS; SOUZA; VENZON, 2002).

The simplification of the cultivation, that is normally and widely applied in Brazil, also contribute to a better environment for other species to reach economic importance, as cicadas, with emphasis on *Quesada gigas* (Hemiptera: Cicadidae) (Oliv., 1790), which is the species that causes the most damage. But also, *Dorisiana drewseni* (Hemiptera: Cicadidae) (Stal., 1854), *D. viridis* (Hemiptera: Cicadidae) (Oliv., 1790), *Fidicinoides pronoe* (Hemiptera: Cicadidae) and many others, can cause great damage, especially in a unstable environment. Its damage came from the nymphs that suck the sap from the roots of coffee plants, which can, in some cases, lead to death (SOUZA, 2004).

The population dynamics of these pests varies according to the cultivation regions, due to biotic and abiotic factors that act in the coffee agroecosystem. In relation to biotic factors, natural enemies, especially predators and parasitoids, are important organisms that contribute to the population regulation of these pests (REIS; SOUZA, 2002).

Parasitoids *Phymastichus coffea* (Hymenoptera: Eulophidae) (LaSalle, 1990) and *Heterospilus coffeicola* (Hymenoptera: Braconidae) (Schimideknecht, 1924), both from Africa, are very important natural enemies of beetle borer (HANSON; GAULD, 2006). Also, the

braconids *Orgilus niger* (Penteado-Dias, 1999), *Stiropius letifer* (Mann, 1872), *Eubazus punctatus* (Ratzeburg, 1852) and eulophids *Closterocerus coffeellae* (Ihering, 1914), *Proacrias coffeae* (Ihering, 1914), *Horismenus aeneicollis* (Ashmead, 1904) are identified as important parasitoids of the leaf miner (REIS; SOUZA; VENZON, 2002).

The bethylids *Prorops nasuta* (Waterson, 1923) and *Cephalonomia stephanoderis* (Betren, 1961) are known to be efficient parasitoids of *H. hampei* in Africa for more than 40 years (WALLER; BIGGER; HILLOCKS, 2007) and in Brazil, after its liberation in 1929, *P. nasuta* has been found since 1933 (FERREIRA, A. J., 1980).

Reports by Vega et al. (1999) in work conducted in Ivory Coast, point to *Polynema* sp. (Hymenoptera: Mymaridae), *Tineobius* sp. (Hymenoptera: Eupelmidae), *Chelonus* sp., *Bracon* sp. and *Stenobracon* sp. (Hymenoptera: Braconidae) as potential agents to control coffee borer. Also, Mymaridae are very important egg parasitoids of Auchenorrhyncha (HUBER, 1986; HUBER; VIGGIANI; JESU, 2009) insects that have a potential to cause great damage to the crop (SOUZA, 2004).

Changes in the landscape structure, such as reducing the proportion of fragments of native vegetation or increasing their isolation, can alter the ability of natural enemies to disperse, thus reducing the size of regional populations (JONSEN; FAHRIG, 1997). But, by the other hand, its maintenance can be done through diversification and conservation of the environment. (ALTIERI; SILVA; NICHOLLS, 2003; LANDIS; WRATTEN; GURR, 2000).

### **2.3 Diversification and Conservation Biological Control**

With the increase in the environment's diversity, natural enemies are helped in several ways, mainly: by offering alternative food for adults, such as nectar, pollen and sugary substances; availability of shelter and adequate microclimate and the presence of prey and alternative hosts for natural enemies (ANDOW, 1991; ROOT, 1973). A more diversified coffee culture promotes an increase in the diversity of insects, birds and other organisms that help in the control of possible pests (BORKHATARIA, Rena *et al.*, 2012; PERFECTO *et al.*, 1996; SOTO-PINTO *et al.*, 2000).

The ecosystem can provide a huge variety of services and goods that we, as humans, can benefit from and should benefit. Those services are normally set aside due to common sense of what came from nature is "free" and there are not a price associated with it (DE GROOT, 1987). But, when we take those services in account, we can see it's potential, as showed by

Losey and Vaughan, (2006) the service of pest control conducted by natural enemies contributes to more than 4 billions of dollars in the United States and maintain around 65% of the potential pests under control.

Conservation biological control is based on maintaining natural enemies in the cultivation area using attractive plants, making a complex spatial arrangement, and maintaining native border vegetation. These plants will provide, among other things, shelter, and alternative food for natural enemies. This vegetal diversification provides an increase in the number of natural enemies in the area, allowing them to act in the regulation of pest (ALTIERI, 1999; ALTIERI; SILVA; NICHOLLS, 2003; BARBOSA, 1998; ROOT, 1973). This pest regulation can contribute to an mortality of up to 80% of the leafminer insect (PEREIRA *et al.*, 2007).

With the increase in the diversity of the environment, natural enemies are helped in several ways, especially through the provision of alternative food for adults, such as nectar, pollen and sugary substances; the availability of shelter and adequate microclimate, and the presence of prey and alternative hosts for natural enemies (ALTIERI; SILVA; NICHOLLS, 2003; LANDIS; WRATTEN; GURR, 2000).

In coffee systems, it is known that plant diversification, whether anthropic or natural, promotes a significant increase in the richness of species of natural enemies, especially parasitoids of coffee pests (FERNANDES, 2013; FERREIRA, Fabricio Zelesnikar; SILVEIRA; HARO, 2013; PERIOTO *et al.*, 2004; SANTOS; PÉREZ-MALUF, 2012). In a coffee system, diversified with banana and a tree for shade in Uganda, the parasitism rate and the abundance of parasitoids was increased (IJALA *et al.*, 2019).

Is possible to benefit from the surrounding vegetation as source of natural enemies. It is known that the crop boarder native vegetation improve ecological services such as the action of natural enemies by harbouring a great abundance and diversity of parasitoids (DAINESE *et al.*, 2019). There are many ways to utilize the surrounding vegetation, as native fragments near the crop, vegetation corridors and, specifically in southern Minas, Valos, all these can increase the diversity of natural enemies and the movement between crop fields (ALTIERI; SILVA; NICHOLLS, 2003). Valos are typical landscape structure found in all south of Minas Gerais state. These structures are composed of a channel sculped on the ground to separate farms on the past, that are now colonized by a great variety of trees and are now treated as vegetation corridors (CASTRO 2004). These valos can contribute with the parasitoid fauna and near it, the presence of parasitoids is increased (GOMEZ, 2007).



Also, in coffee systems, this vegetable diversification can be done using, for example, plants for shading. These provide input of organic matter, nutrients, conserve the soil and can represent an extra source of tree resources for rural family activity (GUHARAY, 2001) and also can contribute economically by the direct or indirect commercialization of the products of the trees (BORKHATARIA, Rena. R.; COLLAZO; GROOM, 2012; KHATOUNIAN, 2001; PERFECTO *et al.*, 1996).

### 2.3 Three species for diversification

Some examples of trees for diversification that can also bring more income and other benefits are the Avocado tree (*Persea Americana* Mill.) originally from Mexico and Central America is fruit tree that grows up to 20 meters with a dense canopy and intense flowering. It has a high production potential with yields reaching 140 kg/tree per year at seven years old and a great variety of uses (DUARTE *et al.*, 2016). Its flowers can attract a great variety of insects parasitoids, specially from families Braconidae, Bethylidae, Eulophidae, Eulpeimidae, Pteromalidae and Encyrtidae (PEÑA *et al.*, 2015) and also are very effective in attract predators such as wasps (TOMAZELLA *et al.*, 2018) and insects from Anthocoridae family (PEÑA *et al.*, 2015).

Mangium (*Acacia mangium* Wild) is a tree originated from northwest Australia and surroundings. It has a fast-grown rate and can live up to 40 years and can reach heights of 30 meters has a dense canopy and intense flowering. Also, it makes symbiosis with some Rhizobium (REDDELL; WARREN, 1986). It has a huge variety of utilizations, such as: Wood, Reforestation, honey tree, and energy (MACKEY, 1996).

Red Cedar (*Toona ciliata* M. Roem.) is forest tree originated from south Asia and Australia with fast grown rate. It can reach up to 30 meters height and its primary use is for timber (MAUNDU; TENGNÄS, 2005).

The Macadamia tree (*Macadamia tetraphylla* L.A.S.Johnson) originally from Australia is a specie with dense foliage that can grows up to 18 meters, has intense flowering, up to 300 flowers per raceme, and high yields(AUGSTBURGER *et al.*, 2000). The nut is very profitable and well accepted by its consumers and it nut shell can be used as coal (PENONI *et al.*, 2011).

Teak tree (*Tectona grandis* Linn.) that is a timber tree originated from India, well known in the world and is renowned for its dimensional stability, hard wood, strength, insect resistance and phytochemicals utilization (PANDEY; BROWN, 2000). It is a large deciduous tree that

can reach a height of 30 to 40 meters with broad shiny leaves that can reach 50 centimetres. Teak start flowering around seven years old and its flowering period is on rainy season. The flowers are small, whitish and appear in panicles with a few thousand flower buds and the plant remain with flower for two to four weeks (CHOUGULE; KOUMARAVELOU; NITAVE, 2017). Works conducted by Tangmitcharoen *et al.*, (2006) shows that many parasitoids families are attracted by those flowers such as Braconidae and Bethylidae, and also, may pollinators and predators.

## 2.4 Shaded Coffee

Despite shading in coffee plantation being a very old practice, due to genetic improvement, it was regularly grown in full sun, even though, growing coffee in shade brings great benefits to culture and the environment (KHATOUNIAN, 2001; MANCUSO; SORATTO; PERDONÁ, 2013). The use of trees for shading promotes a greater thermal balance of the environment; decreases erosion risks; increases litter and the presence of symbionts; maintains relative humidity at higher levels, promoting greater comfort for plant and animal species; assists in carbon sequestration; and increases the diversity of animals (PERFECTO *et al.*, 1996; SOTO-PINTO *et al.*, 2000).

Shading directly affects the fauna composition of coffee plantations and the interactions between the various organisms present, as the rates of infestation and survival of fruit flies (Tephritidae and Lonchaeidae) are affected, with shading promoting less infestation by the tephritids in shaded coffee, especially of the Catuaí cultivar (AGUIAR-MENDEZ *et al.*, 2007). Furthermore, the occurrence of the coffee borer is also reduced in shaded coffee plantations (JONSSON *et al.*, 2014) and its abundance within the fruits is also lower, as is the male / female ratio in coffee plants in full sun (MARIÑO *et al.*, 2016). Looking at the Hemiptera family, that have a huge variety of insects that can cause great damage to the coffee crop, the presence of shadow affect negatively the presence of those insects, being more abundant on sun coffee (KARUNGI *et al.*, 2015).

Works show that with the shadow came the natural enemies too, in work conducted by Karungi *et al.*, (2015), they found that the population of ants increase as shadow increases and Pak *et al.*, (2015) found that the parasitoid abundance also increase in shaded coffee.

The selection of the tree for diversification and shade have to be very cautious, taking in account many aspects, some simple as size, canopy cover, leaf drop, and some more complex

as, organic matter and competition, but as positive aspect it can contribute to an increase in the income, with direct utilization of that plant (GUHARAY, 2001; MANCUSO; SORATTO; PERDONÁ, 2013) and as well, it allows the product to be commercialized out of the commodity, reaching more attractive prices (BORKHATARIA; COLLAZO; GROOM, 2012).

### **3 GENERAL CONSIDERATIONS**

First, in none of our collects, was found specimens of the Bethyridae family that are possible the two parasitoids of borer beetle, *Prorops nasuta* and *Cephalonomia stephanoderis*, so, no mention on them were given.

In relation to our results, we found that the diversification can improve and by more, have a better effect on the selected families than both native vegetation and monoculture. Regarding to shadow, the shaded coffee improved diversity and abundance, when compared with sun coffee, and by last, when we compared the trees for intercropping, even them, by general, improved the diversity, no tree stand as a better choice.

So, as conclusion and consideration, the diversification of the coffee plantation, taking in account all beneficial aspects, and now the effect on these important families is a great option to the farmer.

As future research to improve this result, we recommend doing the economic viability of some plants to intercrop coffee crop. Also, the evaluation of the microclimate generated by the trees and the migration of the beneficial insects between Valos, Coffee Crop, Trees, and Vegetation Fragment.

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**SECOND PART – ARTICLES**

## **ARTICLE 1 – DIVERSIFIED AND MONOCULTURE, DIFFERENCES IN SOME PARASITOID COMMUNITY OF COFFEE CROP**

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Prepared according model of Neotropical Entomology

### **ABSTRACT**

The diversification of the agriculture is a practice that is getting focus in the last decade, but it always was the natural way. Monocultures are used to improve yield by, mainly, improving the density of plants in a production area. With the simplification of the agriculture come a great loss in the diversity of animals present at the plantation and with this, the loss of important ecological services. The diversification, being anthropomorphic or natural, brings benefits to the crop, farmer, and the environment. One way possible to restore the diversity of the crop, is planting trees along the culture. Doing this, we can restore the diversification and help to improve ecological function, as pest control, and can also, benefit from the tree resources, as timber and fruits. This work was conducted at Fazenda da Lagoa, Santo Antonio do Amparo municipality, Brazil in a six-year-old coffee plantation intercropped with different tree species, being them Avocado, Macadamia, Mangium, Teak and Red Cedar. The insects were collected utilizing yellow plastic pan traps that were left in the field for a 48h period. Each tree species was sampled separated and a full sun coffee as control. We found 254 insects in 40 species being 22 species of Braconidae, six species of Eulophidae and 12 Mymaridae species. Our results showed that all tree species had a greater insect richness than Monoculture coffee with Avocado (16), Mangium (18), Cedar (16), Macadamia (19), Teak (16) and Full Sun (10), also we found more abundance of parasitoids at the diversified coffee than in monoculture. We haven't found difference between the tree species used for diversification, but, in overall the diversification brought more diversity of parasitoids to the crop.

**KEYWORDS:** Braconidae; Eulophidae; Mymaridae; Diversification; Avocado; Teak; Mangium; Cedar; Macadamia.

### **1 INTRODUCTION**

Conservation biological control is based on the maintenance of natural enemies in cultivation by enhancing its population and given condition to them to maintain at the local. This is possible utilizing the natural vegetation of the surroundings or by the diversification of the cultivation. The plants for diversification should provide, among other things, shelter and alternative food for natural enemies and with this, an increase in the number of natural enemies in the area will occur, allowing them to act in the regulation of pest insects (Root 1973; Barbosa 1998; Altieri 1999; Altieri et al. 2003).

This diversification also promotes shadow and other benefits for the crop and the soil, even the region is affected. Some beneficial characteristics are the more stable micro climate, lit cover, organic matter, temperature, alternative food for natural enemies, alternative food for some potential pests, impeding it to become pests (Guharay 2001; Letourneau et al. 2011; Nicholls and Altieri 2013).

Coffee is a perennial plant from *Coffea* L. genus, having more than hundred species worldwide and can, by its own, harbour a great variety of parasitoids and other insects as found out by some authors, as Melo et al. (2007); Perioto et al.(2011); Ferreira et al. (2013); Tomazella et al. (2018). But, a more diversified coffee culture promotes an increase in the diversity of insects, birds and other organisms that help in the control of possible pests (Perfecto et al. 1996; Soto-Pinto et al. 2000; Perfecto et al. 2003; Borkhataria et al. 2012b). As a perennial crop, in coffee plantation, these diversity can be enhanced utilizing trees to intercrop coffee, what can else contribute to the enhancement of organic matter, nutrients, soil conservation and alternative food for natural enemies (Guharay 2001).

And is well know that the diversification of the coffee crop being anthropic or natural, promotes a significant increase in the richness of species of natural enemies, especially parasitoids of coffee pests (Perioto et al. 2004; Santos and Pérez-Maluf 2012; Fernandes 2013; Ferreira et al. 2013). Many natural enemies, especially parasitoids, are very important and efficient at pest regulation. Parasitoids from families Braconidae, Eulophidae, Bethylidae and Mymaridae have an important role in this, having many species that control the Coffee Beetle Borer, Leaf Miner and many leafhoppers and cicadas (Penteado-Dias 1999; Vega and Kirkl 1999; Reis and Souza 2002; Hanson and Gauld 2006; Roberto et al. 2013).

Besides the ecological benefit, the diversification can have an economic benefit, by the exploit of the plant used for diversification, as some trees, that can produce fruits and/or lumber (Perfecto et al. 1996; Khatounian 2001; Borkhataria et al. 2012a).

Some trees that can be used for diversification that have an economic viability are, for example, Avocado tree (*Persea Americana* Mill.) originally from Mexico and Central America is fruit tree that grows up to 20 meters with a dense canopy and intense flowering. It has a high production potential with yields reaching 140 kg/tree per year at seven years old and a great variety of uses (Duarte et al. 2016).

Mangium (*Acacia mangium* Wild) is a tree originated from northwest Australia and surroundings. It has a fast-grown rate and can live up to 40 years and can reach heights of 30 meters has a dense canopy and intense flowering. Also, it makes symbiosis with some

Rhizobium (Reddell and Warren 1986). It has a huge variety of utilizations, such as: Wood, Reforestation, honey tree, and energy (Mackey 1996).

Red Cedar (*Toona ciliata* M. Roem.) is forest tree originated from south Asia and Australia with fast grown rate. It can reach up to 30 meters height and its primary use is for timber (Maundu and Tengnäs 2005).

The Macadamia tree (*Macadamia tetraphylla* L.A.S.Johnson) originally from Australia is a specie with dense foliage that can grows up to 18 meters, has intense flowering, up to 300 flowers per raceme, and high yields(Augstburger et al. 2000). The nut is very profitable and well accepted by its consumers and it nut shell can be used as coal (Penoni et al. 2011).

Teak tree (*Tectona grandis* Linn.) that, is a timber tree originated from India, well known in the world and is renowned for its dimensional stability, hard wood, strength, insect resistance and phytochemicals utilization (Pandey and Brown 2000). It is a large deciduous tree that can reach a height of 30 to 40 meters with broad shiny leaves that can reach 50 centimetres. Teak start flowering around seven years old and its flowering period is on rainy season. The flowers are small, whitish and appear in panicles with a few thousand flower buds and the plant remain with flower for two to four weeks (Chougule et al. 2017).

The effect of this consortiation is poorly known so far and thus our goal was to find out if the trees used to diversification can enhance the diversity, richness, and abundance of Braconidae, Eulophidae, Mymaridae and Bethyilidae parasitoid families when compared with a monoculture coffee.

## 2 MATERIAL AND METHODS

The present work was conducted at Fazenda da Lagoa Km 642 of BR 381, municipality of Santo Antônio do Amparo, Minas Gerais, Brazil (20°91'S/44°85'W/1100m) in a coffee plantation (*Coffea arabica* L.) cultivar Catuaí 99, six years old, landed by the farm to this experiment. Were utilized five plots of intercropped coffee with different tree species and a monoculture coffee as control. The tree species utilized were Avocado (*Persea americana* Mill.), Mangium (*Acacia mangium* Willd.), Cedar (*Toona ciliata* M. Roem.), Macadamia (*Macadamia tetraphylla* L.A.S.Johnson) and Teak (*Tectona grandis* L.f).

Each plot of two hectares consisted of coffee crop, grown at 3.40m x 0.65m spacing, conducted in the conventional cultivation system with total control of spontaneous plants, leaving the crop always clean. The cultural treatments, as well as the harvest, were carried out

mechanically. The control of pests and diseases was carried out following the premises of the IPM, in which a monthly survey of the occurrence of pests was carried out and, after evaluation, the appropriate decision was made. If control was necessary, they performed insecticide applications.

The companion species were cultivated in the same period, therefore having the same age as the coffee plantation. However, due to physiological characteristics, they differed in size and phenological period. In each plot the tree species were cultivated in the same row as coffee being planted seven meters from each other in a row and 16 meters from each row of trees.

The collections were carried out quarterly from June / 2016 to March / 2018, totalling eight collections, using yellow plastic traps, with 20cm in the largest diameter and 10cm in the smallest, suspended 50cm from the floor affixed to a piece of bamboo. The traps were left in the field for 48 hours, containing a 10% saline solution of NaCl and neutral detergent.

Within each plot, six representative sample points were selected. At each point, a trap was installed, each point being at least 50 meters apart. The insects collected were kept in alcohol 70 % and identified up to the family level according to didactic material by Goulet and Huber (1993) and separated into morphospecies and subsequently identified up to genus and species (Figure 1).

Figure 1 – Aerial view of the experiment area with delimitation of each treatment showing yellow dots representing the yellow pan traps utilized. Santo Antônio do Amparo, Brazil, 2018.



For data evaluation, the following analyses were made using the software R Studio (RSTUDIO TEAM, 2016), PAST® (Hammer, Harper, & Ryan, 2001), and Microsoft Excel 365 and the data were transformed to  $\sqrt{(x + 0.5)}$ , when necessary.

1) Rarefaction curves of species collected according to Coleman, (1981), which allow us to conclude whether the samples were regular and sufficient to collect, potentially, all species that occur in the culture.

2) Bootstrap richness estimator, which uses data from all species collected to estimate total richness, not being restricted to rare species (Efron and Tibshirani 1993).

3) Species richness (S), which is the total number of species or morphospecies collected.

4) Abundance index, according to Lamshead, Platt, & Shaw, (1983), calculated from the means of each species per sample.

5) H' diversity index, according to Shannon & Weaver, (1949), which takes into account the quantitative uniformity of each species in relation to the others.

### 3 RESULTS

We found 254 insects in 40 species being 22 species of Braconidae, six species of Eulophidae and 12 Mymaridae species (Table 1) and no Bethyridae was found, with overall bootstrap of 44.59 and a richness of 40 we have a significance value of 89.71%. Along these insects collected, 152 was from Braconidae family, 18 from Eulophidae and 84 from Mymaridae representing 59.84%, 7.08% and 33.07% respectively.

Macadamia treatment was the highest for Braconidae, being found 23.02% of all braconids, followed by Mangium with 19.73%. The treatment with less Braconidae was Cedar, with 11.84% of all Braconidae. For Mymaridae the treatment that harboured more was Mangium with 33.33%, followed by Monoculture, with 27.77% of all individuals. The treatment with less Mymaridae was Avocado, with 0. And for Eulophidae, Cedar, with 30.09% was the highest, followed by Teak with 21.42% and the less abundant was Monoculture, with only 3.57%.

At Avocado, 73.5% of all insects reared were Braconidae, 26.5% was Mymaridae and no Eulophidae was found. At Mangium, 56.6% was Braconidae, 11.3% was Eulophidae and 32.1% was Mymaridae. At Cedar, 39.1% was Braconidae, 4.3% was Eulophidae and 56.5 was Mymaridae. For Macadamia the results were 70% Braconidae, 8% Eulophidae and 22% Mymaridae. At Teak was found 56.8% for Braconidae, 2.3% for Eulophidae and 40.9% of Mymaridae. And at least, for Monoculture was found 70.4 % Braconidae, 18.5% Eulophidae and 11.1% Mymaridae.

Table 1 – Abundance (X) and frequency (%) of taxons sampled from coffee shaded with different trees and at Monoculture, corresponding to the families Bra = Braconidae; Eul = Eulophidae and Mym = Mymaridae. Santo Antonio do Amparo, Brazil, 2018.

Taxon	Avocado		Mangium		Cedar		Macadamia		Teak		Monoculture	
	X	%	X	%	X	%	X	%	X	%	X	%
<b>Braconidae</b>												
1. Bra sp.1	-	-	6	11.3	6	13.0	21	42.0	10	22.7	6	22.2
2. Bra sp.2	6	17.6	1	1.9	-	-	2	4.0	-	-	-	-
3. Bra sp.3	3	8.8	6	11.3	5	10.9	-	-	1	2.3	5	18.5
4. Bra sp.4	2	5.9	3	5.7	-	-	-	-	-	-	-	-
5. Bra sp.6	-	-	3	5.7	1	2.2	-	-	-	-	-	-
6. Bra sp.9	-	-	-	-	-	-	2	4.0	-	-	-	-
7. Bra sp.11	-	-	-	-	1	2.2	-	-	-	-	5	18.5
8. Bra sp.12	4	11.8	4	7.5	2	4.3	-	-	2	4.5	-	-
9. Bra sp.13	-	-	-	-	1	2.2	-	-	-	-	-	-

Table 1 - Continuing

Taxon	Avocado		Mangium		Cedar		Macadamia		Teak		Monoculture	
	X	%	X	%	X	%	X	%	X	%	X	%
10. Bra sp.14	2	5.9	1	1.9	1	2.2	-	-	2	4.5	-	-
11. Bra sp.15	4	11.8	-	-	-	-	6	12.0	3	6.8	3	11.1
12. Bra sp.16	1	2.9	-	-	-	-	1	2.0	-	-	-	-
13. Bra sp.17	-	-	2	3.8	-	-	-	-	-	-	-	-
14. Bra sp.18	1	2.9	-	-	-	-	-	-	-	-	-	-
15. Bra sp.19	-	-	-	-	-	-	2	4.0	1	2.3	-	-
16. Bra sp.20	-	-	-	-	-	-	-	-	2	4.5	-	-
17. Bra sp.22	-	-	-	-	-	-	1	2.0	-	-	-	-
18. Bra sp.25	-	-	4	7.5	-	-	-	-	-	-	-	-
19. Bra sp.26	1	2.9	-	-	-	-	-	-	2	4.5	-	-
20. Bra sp.27	1	2.9	-	-	-	-	-	-	-	-	-	-
21. Bra sp.29	-	-	-	-	-	-	-	-	2	4.5	-	-
22. Bra sp.30	-	-	-	-	1	2.2	-	-	-	-	-	-
<b>Subtotal</b>	<b>25</b>	<b>73.5</b>	<b>30</b>	<b>56.6</b>	<b>18</b>	<b>39.1</b>	<b>35</b>	<b>70.0</b>	<b>25</b>	<b>56.8</b>	<b>19</b>	<b>70.4</b>
<b>Eulophidae</b>												
23. Eul sp.2	-	-	3	5.7	2	4.3	1	2.0	-	-	-	-
24. Eul sp.3	-	-	-	-	-	-	1	2.0	-	-	-	-
25. Eul sp.4	-	-	-	-	-	-	-	-	1	2.3	3	11.1
26. Eul sp.5	-	-	3	5.7	-	-	-	-	-	-	2	7.4
27. Eul sp.6	-	-	-	-	-	-	1	2.0	-	-	-	-
28. Eul sp.8	-	-	-	-	-	-	1	2.0	-	-	-	-
<b>Subtotal</b>	<b>-</b>	<b>-</b>	<b>6</b>	<b>11.3</b>	<b>2</b>	<b>4.3</b>	<b>4</b>	<b>8.0</b>	<b>1</b>	<b>2.3</b>	<b>5</b>	<b>18.5</b>
<b>Mymaridae</b>												
29. Mym sp.1	2	5.9	5	9.4	6	13.0	2	4.0	4	9.1	-	-
30. Mym sp.2	-	-	5	9.4	-	-	1	2.0	-	-	-	-
31. Mym sp.3	-	-	-	-	-	-	1	2.0	-	-	1	3.7
32. Mym sp.4	1	2.9	-	-	9	19.6	-	-	-	-	-	-
33. Mym sp.5	1	2.9	1	1.9	3	6.5	1	2.0	-	-	1	3.7
34. Mym sp.6	-	-	-	-	5	10.9	-	-	-	-	-	-
35. Mym sp.7	-	-	-	-	-	-	2	4.0	4	9.1	-	-
36. Mym sp.8	-	-	-	-	-	-	-	-	1	2.3	-	-
37. Mym sp.9	4	11.8	-	-	2	4.3	2	4.0	3	6.8	1	3.7
38. Mym sp.10	1	2.9	1	1.9	1	2.2	2	4.0	6	13.6	-	-
39. Mym sp.11	-	-	3	5.7	-	-	-	-	-	-	-	-
40. Mym sp.12	-	-	2	3.8	-	-	-	-	-	-	-	-
<b>Subtotal</b>	<b>9</b>	<b>26.5</b>	<b>17</b>	<b>32.1</b>	<b>26</b>	<b>56.5</b>	<b>11</b>	<b>22.0</b>	<b>18</b>	<b>40.9</b>	<b>3</b>	<b>11.1</b>
<b>TOTAL</b>	<b>34</b>	<b>100.0</b>	<b>53</b>	<b>100.0</b>	<b>46</b>	<b>100.0</b>	<b>50</b>	<b>100.0</b>	<b>44</b>	<b>100.0</b>	<b>27</b>	<b>100.0</b>



Looking at the Richness values (Table 2), we found 16 species at Avocado, 18 at Mangium, 16 at Cedar, 19 at Macadamia, 16 at Teak and 10 at Monoculture, with sampling significance of 89%, 94%, 90%, 85%, 93% and 97% respectively. For diversity we found that Mangium was the more diverse with  $H'$  of 0.57 and Monoculture, the less diverse with  $H'$  of 0.07.

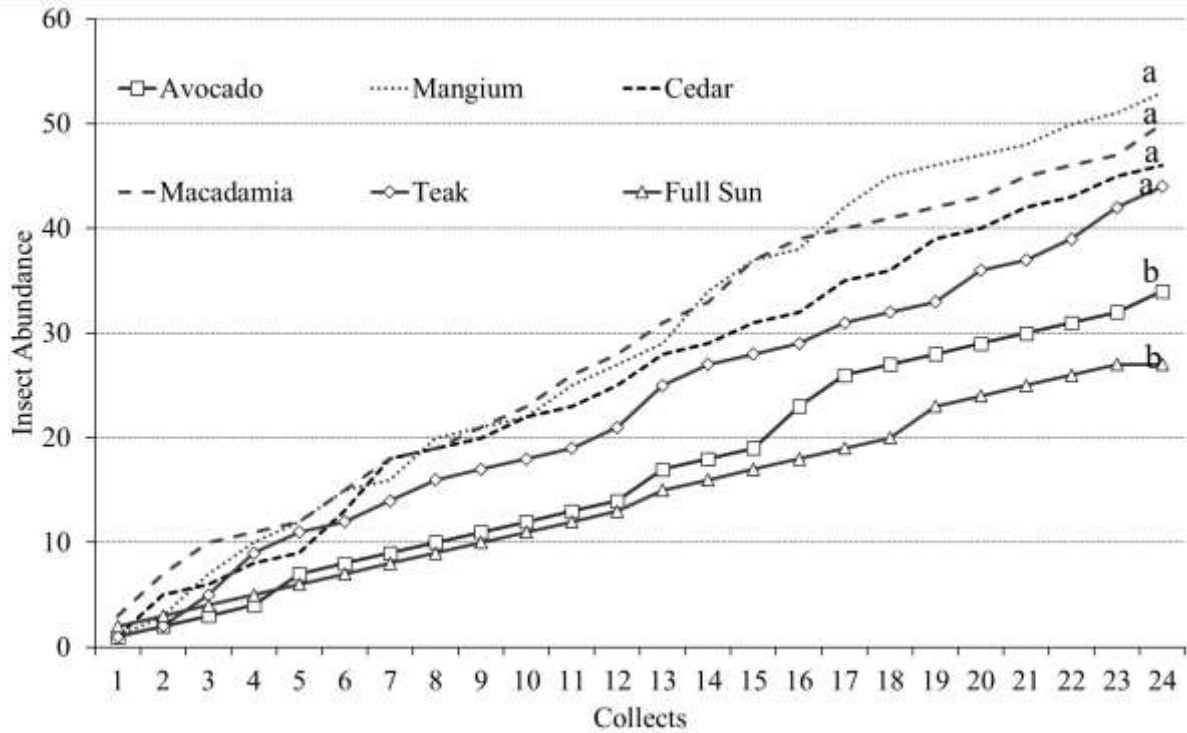
Table 2 – All index for all treatments with letters indication statistical difference. Percentage index is a representative for the efficiency of sampling calculate dividing Bootstrap by Richness. Santo Antônio do Amparo, Brazil, 2018.

Index	Avocado	Mangium	Cedar	Macadamia	Teak	Monoculture
<b>Abundance</b>	34 b	53 a	46 a	50 a	44 a	27 b
<b>Bootstrap</b>	18	19.05	17.83	22.35	17.23	10.3
<b>Richness</b>	16 a	18 a	16 a	19 a	16 a	10 b
<b>Percentage</b>	89%	94%	90%	85%	93%	97%
<b>Diversity <math>H'</math></b>	0.19 b	0.57 a	0.42 a	0.38 a	0.32 a	0.07 b

\*Different letters in a row mean different values according to Scott-Knott test at 5% significance.

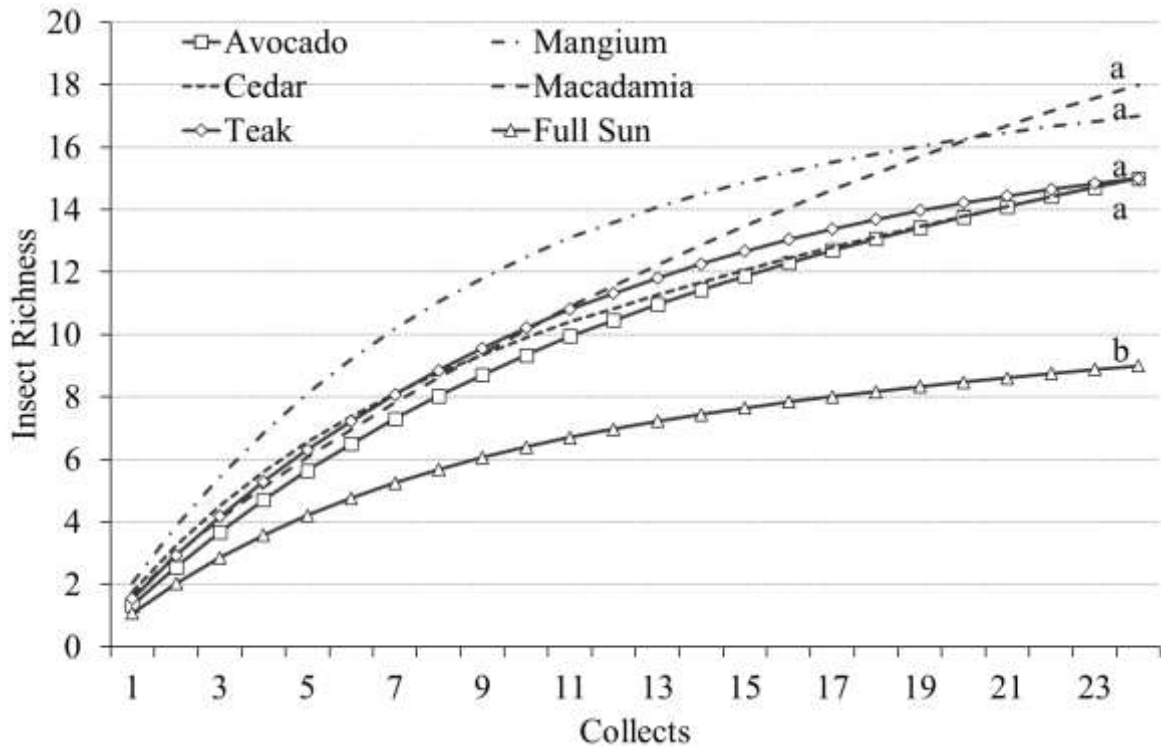
When we take a look at the abundance over time, in other words, the insects sampled in each collect grouped together, it's possible to see that Mangium started with lower insects than all other treatments, except Monoculture (Figure 2), and stayed similar to Macadamia for a long time, but at the 17<sup>a</sup> collect, it became higher and stayed like this. Avocado and Monoculture expressed similar patterns along the time with avocado being higher at the end, but, even so, both had lower abundance than other treatments. At the end, was possible to see that all treatments start to get far from each other.

Figure 2 - Number of individuals by time in all different treatments with small letters showing statistical difference according to Scott-Knott test at 5%. Santo Antônio do Amparo, Brazil, 2018.



As possible to see at Figure 3, for specie richness, the treatment Monoculture had the lowest value from the beginning to the end of the collects ending with only 10 species collected. Mangium had a huge difference from others at the beginning, but, at the end with richness value of 18, it started to become stagnant and, ended with lower value than Macadamia with 19. By the other hand, Macadamia started with similar values of Cedar, Teak and Avocado, but by the middle, it started to become richer. Cedar, Teak and Avocado shared the same richness of 16 at the end and the same pattern through time.

Figure 3 – Species Richness by time in all treatments with small letters showing statistical difference according to Scott-Knott test at 5%. Santo Antônio do Amparo, Brazil, 2018.



#### 4 DISCUSSION

According to Efron and Tibshirani (1993) the bootstrap estimator show the potential of sampling and when you compare this with the richness found, we can tell if the sampling were enough or not. By average, a relation of 80% or more is considered efficient, so, here we can consider that the collects were enough. Having overall bootstrap of 44.59 and a richness of 40 we have a percentage of 89.71 % and individuals of 89% for Avocado; 94% for Mangium; 90% for Cedar; 85% for Macadamia; 93% for Teak and 97% for Monoculture. We can consider that all sampling was enough (Table 2). Taking that in account, we can look to possible differences between treatments.

As the sampling sites were distant of each other by more than 100, we can consider they as isolated treatments as traps have a efficiency range of at most 50 meters (Gomez 2007) and so, we can do some statistical analysis as analysis of variance. With this, we found that for Abundance and Diversity, Avocado and Monoculture treatment showed similar results being different from the other treatments.

According to Perioto et al. 2002; Fernandes 2013; Ferreira et al. 2013; Tomazella 2016, coffee can harbour a great variety of parasitoids, no matter where it is cultivate in Brazil and its surroundings, coffee host 26 parasitoid families, meaning that there is enough food, shelter and other essential need to all of it, but, when we look at the important families, it don't shows the same result. Avocado is a tropical tree with dense canopy and great flowering period (Falcão et al. 2001) , that can harbour and sustain a great variety and quantity of insects like, bees (Falcão et al. 2001) and wasps (Tomazella et al. 2018).

With greater resource offering, the overall abundance of all insects tend to be greater, but, individual abundance tends to be lower, as more species share the same resources (Verberk 2012), meaning that if we had collected all insects in this treatment it possible would be greater than any other, but, as we only worked with four families, the abundance of each was lower. By this, it is not strange that it is grouped with Monoculture treatment.

When we look at Richness, we can see that the Monoculture treatment was lower than others, meaning that diversification was responsible to this increase, as expected according to diversification theories proposed by Root (1973); Andow and Risch (1985); Andow (1991) so with this, we expected to find some difference between plants. Even though no difference was found, Mangium had the greater Abundance, Richness and Diversity, Followed by Macadamia and Cedar.

For Mangium to host a great abundance of parasitoids, was expected, as this tree have an intense flowering (Reddell and Warren 1986), meaning plenty of resources for parasitoids to live and also, this tree is host for a great number of insects from Lepidoptera family (Lunz et al. 2011) a family that has lots of hosts for Braconidae, Eulophidae and Mymaridae parasitoid families (Hanson and Gauld 2006). The Macadamia, also has an intense flowering and produces great quantities of pollen and nectar (Augstburger et al. 2000) also, it can harbour nearly 28 families of Hymenoptera wasps being the great majority from parasitoids families (Makim and Carr 2020).

Red Cedar do not have an intense flowering, but is highly attacked by *Hypsipyla robusta* (Moore, [1886]) a moth from the Pyralidae family (Cunningham and Floyd 2006) that do not occur in Brazil, but, here we have relates of *Hypsipyla grandella* Zeller damaging the seeds (Castro et al. 2017) and as a lepidoptera, it have many parasitoids from the studied families. And for Teak, it has a great variety of pollinators and its flowers can attract a huge variety of Lepidoptera (Tangmitcharoen et al. 2006) and many Hymenoptera (Paes et al. 2014).

With this, we can conclude that, utilize Avocado, Mangium, Cedar, Macadamia and Teak to diversify coffee crop have a good impact on abundance and richness of the Braconidae, Eulophidae and Mymaridae families of parasitoid wasps, along all other know benefits of diversification. Also, those plants have an economic viability and can act as refuge for the beneficial insects and also improve the value of the plantation.

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## **ARTICLE 2 – SHADED AND SUNNY COFFEE: CONTRASTS IN SPECIES OF IMPORTANT PARASITOID FAMILIES**

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Prepared according model of Neotropical Entomology

### **ABSTRACT**

Coffee is a very important crop to Brazil, since is the world greatest producer. But its production is mainly under full sun, to increase its productivity and density of cultivation. It happens that coffee is typically produced under tree canopy, in other words, shade. Shaded coffee has an improvement in diversity and other important factors such micro climate, carbon cyclin, thermal equitability, and others. Also, shade promotes a more suitable environment for many animals, such as arthropods, birds, and mammals enhancing its abundance, richness and survival possibility improving the pest control service promoted by those animals. But, unfortunately, shade coffee is a disappearing system in Brazil with great loss of diversity. With this in mind, this work aimed to see the differences promoted by shadow in the community of four important parasitoids families. This work was conducted at Fazenda Bela Vista (21°04'S;45°04'W; 1070m), Perdões municipality state of Minas Gerais, Brazil in a coffee plantations, of eight hectares, cultivar Catucaí and 20 years old, planted at 3.4m x 0.65m spacing, conducted in a conventional cultivation system, shaded and other coffee plantation of eight hectares, cultivar Catucaí and 20 years old, planted at 3.4m x 0.65m spacing, conducted in a conventional cultivation system at Full Sun. The insects were sampled utilizing yellow pan traps that stayed at the field for 48 hours. We found 99 individuals belong to Braconidae, Eulophidae and Mymaridae families, being 13 morphospecies from Braconidae, five from Eulophidae and two from Mymaridae. Also, the Shadow treatment harboured 57 insects, while the Sun treatment harboured 42 insects and was possible to see a great difference between the communities of Shaded and Sun coffee, with Shaded more diverse than Sun.

**KEYWORDS:** Braconidae, Eulophidae, Mymaridae, Conservation Biological Control

### **1 INTRODUCTION**

Coffee is a crop plant originally grow under canopy cover, in other words, in shade conditions, but in Brazil, coffee is cultivated under sun monocultures (Matiello et al. 2010). Being cultivate under full sun conditions imply in a great loss of biodiversity as mammals (Daily et al. 2003), birds (Gleffe et al. 2006) and insects (Perfecto et al. 2003; Mas and Dietsch 2004; Jha and Vandermeer 2010) are less abundant and diverse in unshaded coffee plantations. With this loss, came a decrease in an very important ecological service, the pest control



(Tschardt et al. 2005). Many studies showed that this service is very important and significant in coffee crop, being ants, birds and lizards agents of these services (Perfecto et al. 2004; Borkhataria et al. 2006; De la Mora et al. 2008; Philpott et al. 2009). And the presence of ants increase with shade in coffee plantations (Moya-Raygoza and Martinez 2014; Karungi et al. 2015). Along this, coffee grow under shadow, provide many benefits and also help to preserve the environment as refuge islands for many animals and plants (Borkhataria et al. 2012a).

Works shows that the control of one of the most important coffee pests, Coffee berry borer (*Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae)) is more effective in coffee shaded plantations than in plantation with lower levels of shade (Teodoro et al. 2008; Karp et al. 2013; Railsback and Johnson 2014) and works conducted by Jaramillo et al. (2013) and Jonsson et al. (2014) found higher berry borer infestation in sun coffee.

One of the main agent of pest control in coffee crop are the parasitoids wasps, that can reach up to 80% level of control (Pereira et al. 2007) and some parasitoids as *Phymastichus coffea* (Hymenoptera: Eulophidae) (LaSalle, 1990) and *Heterospilus coffeicola* (Hymenoptera: Braconidae) (Schimideknecht, 1924), are very important natural enemies of beetle borer (Hanson and Gauld 2006). Also, *Orgilus niger* (Penteado-Dias, 1999), *Stiropius letifer* (Mann, 1872), *Closterocerus coffeellae* (Ihering, 1914) and *Proacrias coffeae* (Ihering, 1914) are identified as important parasitoids of the leaf miner (REIS et al. 2002).

The bethylids *Prorops nasuta* (Waterson, 1923) and *Cephalonomia stephanoderis* (Betren, 1961) are known to be efficient parasitoids of *H. hampei* in Africa for more than 40 years (Waller et al. 2007) and in Brazil, after its liberation in 1929, *P. nasuta* has been found since 1933 (Ferreira 1980). Reports by Vega et al. (1999) in work conducted in Ivory Coast, point to *Polynema* sp. (Hymenoptera: Mymaridae), *Tineobius* sp. (Hymenoptera: Eupelmidae), *Chelonus* sp., *Bracon* sp. and *Stenobracon* sp. (Hymenoptera: Braconidae) as potential agents to control coffee borer. Also, Mymaridae are very important egg parasitoids of Auchenorrhyncha (Huber 1986; Huber et al. 2009) insects that have a potential to cause great damage to the crop (Souza 2004).

The utilization of trees for shade at coffee crop benefit the parasitoid community (Pak et al. 2015) and with this, help the conservation biological control enhancing pest control ecological service. Furthermore, the use of trees for shading also promotes a greater thermal balance of the environment; decreases risks with erosion; increases litter and the presence of symbionts; maintains relative humidity at higher levels, promoting greater comfort for plant

and animal species; assists in carbon sequestration; and increases the diversity of animals (Perfecto et al. 1996; Soto-Pinto et al. 2000). Moreover, the farmer can benefit from shaded coffee by selling it out of commodity price, by commercializing the tree or its products (Khatounian 2001), carbon sequestration (Tschardt et al. 2011).

There were no works conducted in Brazil regarding shade affecting parasitoid families. Taking this in mind, this work aim was to evaluate if a shaded coffee crop can harbour higher abundance, richness and diversity of the parasitoids families Braconidae, Eulophidae, Mymaridae and Bethyridae than sun conducted coffee.

## 2 MATERIAL AND METHODS

The experiment was conducted at Fazenda Bela Vista (21°04'S;45°04'W;1070m), Perdões municipality state of Minas Gerais, Brazil in a coffee plantations, of eight hectares, cultivar Catucaí and 20 years old, planted at 3.4m x 0.65m spacing, conducted in a conventional cultivation system, shaded with Canafístula (*Peltophorum dubium* (Spreng.) Taub. (Fabales: Fabaceae) that formed our Shaded Coffee treatment and other coffee plantation of eight hectares, cultivar Catucaí and 20 years old, planted at 3.4m x 0.65m spacing, conducted in a conventional cultivation system at Full Sun, and this one was our Full Sun treatment.

In each site were selected six representative plots (Figure 1) were have been installed a yellow pan trap with 150 ml of a saline solution of NaCl at 10% and detergent drops.

The sampling was realized each three months for two years, with a total of eight collects. The insects collected from yellow pan traps were washed and kept in alcohol 70 % for identification at the Laboratório de Controle Biológico Conservativo of Universidade Federal de Lavras. All insects were identified up to the family level according to didactic material by Goulet and Huber (1993) and separated into morphospecies.

Figure 1 - Aerial view of the experiment area with delimitation of each treatment showing yellow dots representing the yellow pan traps utilized. Perdões, Brazil, 2018.



For data evaluation, the following analyses were made using the software R Studio (RSTUDIO TEAM, 2016), PAST® (Hammer, Harper, & Ryan, 2001), and Microsoft Excel 365 and the data were transformed to  $\sqrt{x + 0.5}$ , when necessary.

1) Rarefaction curves of species collected according to Coleman, (1981), which allow us to conclude whether the samples were regular and sufficient to collect, potentially, all species that occur in the culture.

2) Bootstrap wealth estimator (or one that is more appropriate), which uses data from all species collected to estimate total wealth, not being restricted to rare species.

3) Species wealth (S), which is the total number of species and morphospecies collected.

4) Abundance index, according to Lamshead, Platt, & Shaw, (1983), calculated from the means of each species per sample.

5) H' diversity index, according to Shannon & Weaver, (1949), which takes into account the quantitative uniformity of each species in relation to the others.

6) NMDS analysis (non-metric multidimensional scaling) (Hennebert and Lees 1991), which graphically shows the difference in similarity between treatments.

7) Similarity analysis (ANOSIM) (Clarke 1993) consists of a non-parametric test to indicate a significant difference between two or more groups based on any measure of distance.

In the case of this work, the Bray-Curtis measure will be used, as it is a robust similarity measure to work with abundance data (Bray and Curtis 1957).

8) Analyse SIMPER (Similarity Percentage), which is a simple method to assess which species are primarily responsible for the difference found between the sampled groups (Clarke 1993).

### 3 RESULTS

We found a total of 99 insects in 20 morphospecies, being 13 from Braconidae family, 5 from Eulophidae family and 2 from Mymaridae family (Table 1), no exemplars from Bethylidae family was found. Braconidae was the most collected family with 84 individuals, corresponding to 84.85% of all insects collected, followed by Eulophidae with 12 corresponding to 12.12% of all insects collected and Mymaridae with only 3, corresponding with 3.03%.

The Shadow treatment was more abundant than Sun, with 57 insects, corresponding to 57.58% of all insects collected. The great majority of these were Braconidae, corresponding to 92.98 of all insects collected at Shadow. Also, only two Eulophidae were found and 2 Mymaridae.

The Sun treatment had more Eulophidae than Shadow, with 83.33% of all Eulophidae sampled and corresponding to 23.81% of all insects collected at Sun treatment.

Table 1 – Abundance (X) and frequency (%) of taxons sampled from shaded coffee and at full sun, corresponding to the families Bra = Braconidae; Eul = Eulophidae and Mym = Mymaridae. Perdões, 2018.

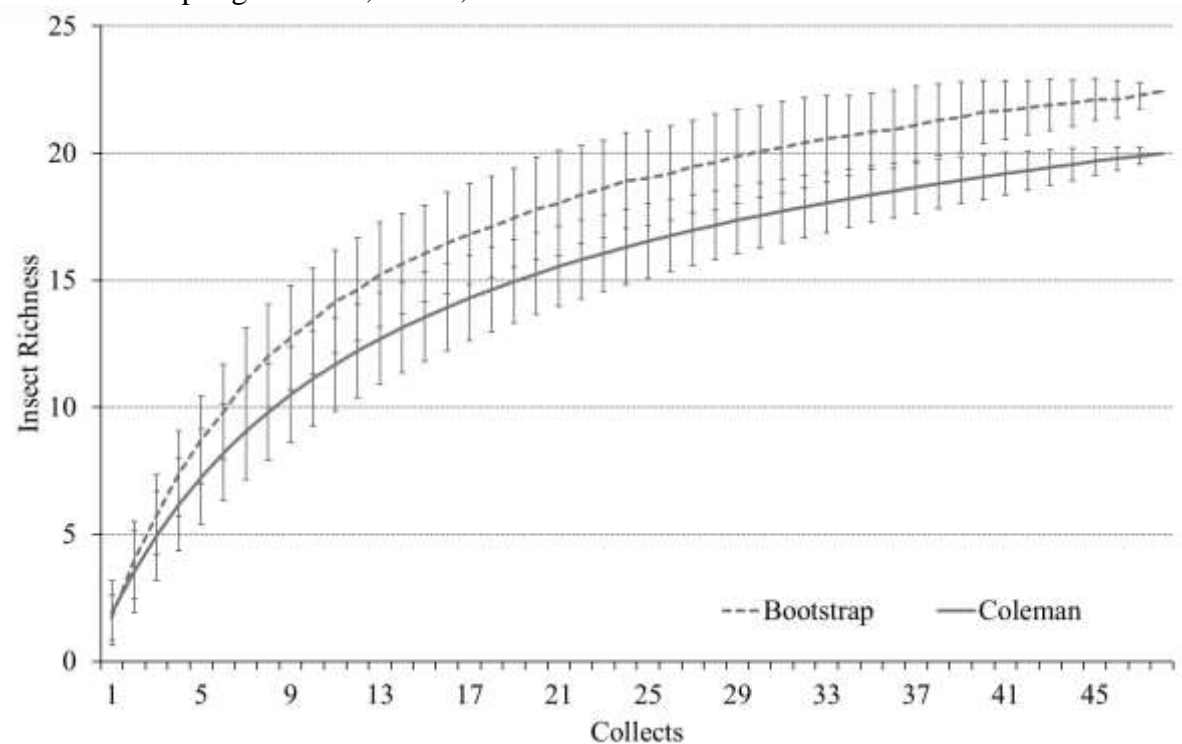
Taxon	Sun		Shadow	
	X	%	X	%
<b>Braconidae</b>				
1. Bra sp.1	3	7.14	11	19.29
2. Bra sp.2	1	2.38	2	3.51
3. Bra sp.3	13	30.95	2	3.51
4. Bra sp.4	2	4.76	2	3.51
5. Bra sp.5	-	-	6	10.53
6. Bra sp.6	4	9.52	6	10.53
7. Bra sp.7	-	-	2	3.51
8. Bra sp.11	1	2.38	-	-
9. Bra sp.15	1	2.38	4	7.02
10. Bra sp.20	2	4.76	4	7.02
11. Bra sp.22	-	-	1	1.75
12. Bra sp.24	4	9.52	8	14.04
13. Bra sp.25	-	-	5	8.77
<b>Subtotal</b>	<b>31</b>	<b>73.81</b>	<b>53</b>	<b>92.98</b>
<b>Eulophidae</b>				
14. Eul sp.1	3	7.14	-	-

Table 1 - Continuing

Taxon	Sun		Shadow	
	X	%	X	%
15. <i>Eul sp.2</i>	1	2.38	-	-
16. <i>Eul sp.3</i>	1	2.38	-	-
17. <i>Eul sp.4</i>	2	4.76	-	-
18. <i>Eul sp.7</i>	3	7.14	2	3.51
<b>Subtotal</b>	<b>10</b>	<b>23.81</b>	<b>2</b>	<b>3.51</b>
<b>Mymaridae</b>				
19. <i>Mym sp.3</i>	1	2.38	-	-
20. <i>Mym sp.12</i>	-	-	2	3.51
<b>Subtotal</b>	<b>1</b>	<b>2.38</b>	<b>2</b>	<b>3.51</b>
<b>Total</b>	<b>42</b>	<b>42.42%</b>	<b>57</b>	<b>57.58%</b>

We found 20 species and our bootstrap predicted 22.43 species, with this our sampling efficiency was 89.16%. This efficiency can be observed in a graphical illustration (Figure 2) grouping the Bootstrap curve with Coleman rarefaction curve were both curves tend to stabilize and don't touch at the final.

Figure 2 – Graphic representation of bootstrap and Coleman's curve with standard errors of all sampling. Perdões, Brazil, 2018.



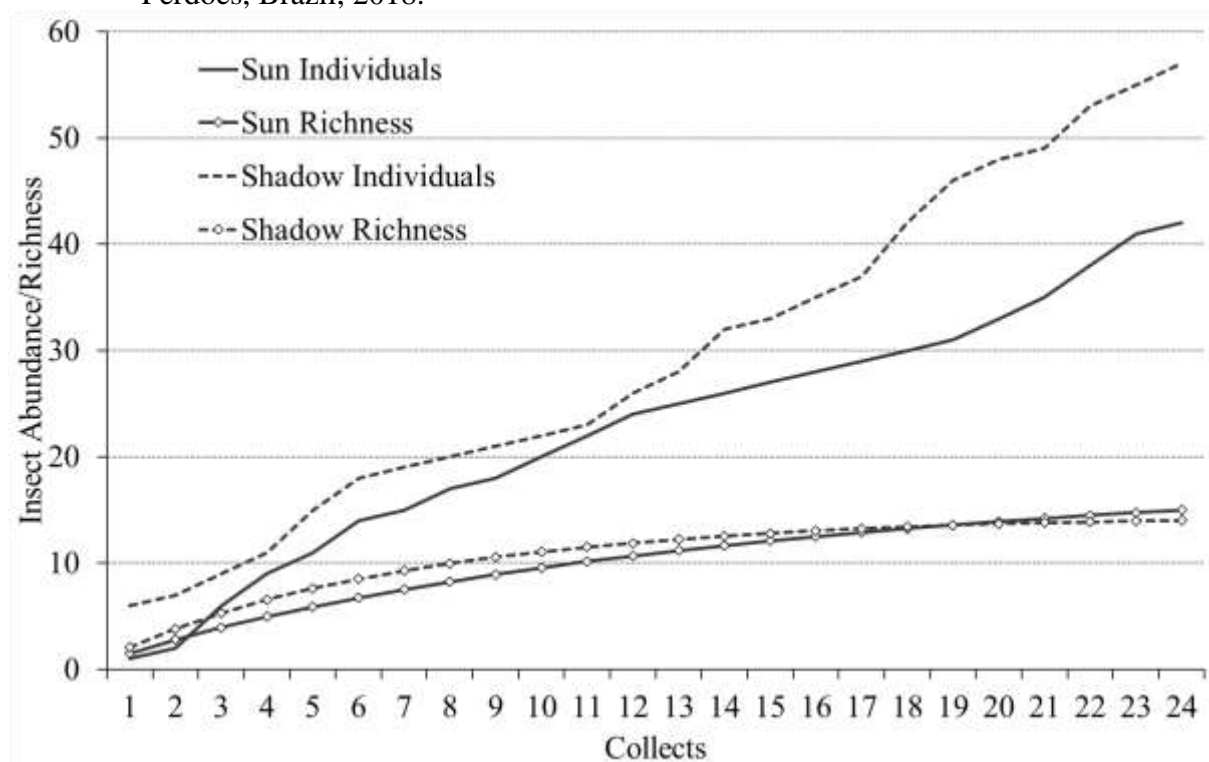
The insect richness for both treatments were similar, being found 15 species at Sun treatment and 14 species at Shadow treatment (Table 2) from a total of 20 species. This represents that Sun had 75% of the possible species found and Shadow 70%. Regarding diversity, Shadow had a value of 0.59 and Sun 0.32.

Table 2 – Table showing Insect Abundance (Abundance), Species Richness (Richness) and Diversity in both treatments with small letters representing statistical difference by Scott-Knott test at 5% significance. Perdões, Brazil, 2018.

Index	Sun	Shadow
Abundance	42b	57a
Richness	15 n.s.	14 n.s.
Diversity H'	0.32b	0.59a

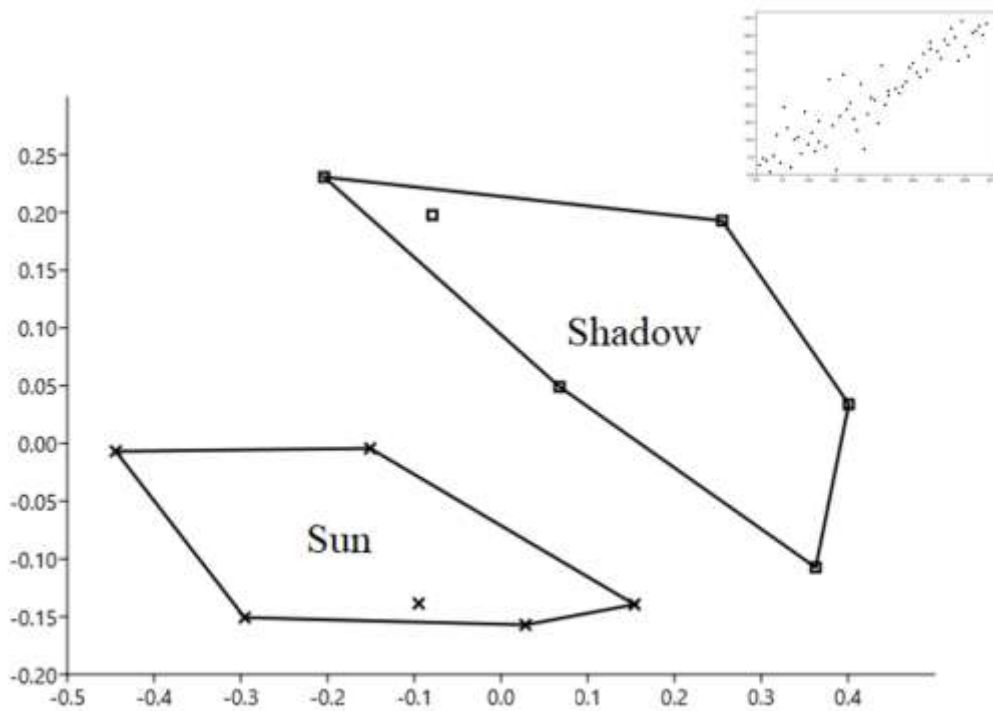
Now, looking at how the abundance and richness grew in time at the collects, we can observe at Figure 3, that the Shadow treatment, from start had always a greater value of abundance than Sun. Richness, in its turn, started very similar for both treatment with Shadow being a little higher, but, from the 20<sup>a</sup> collect, the Sun got higher.

Figure 3 – Graphic representation of Insect Abundance and Species Richness in both treatments. Perdões, Brazil, 2018.



The Non-metric Multidimensional Scaling (NMDS) is a graphic visualization of possible groups formation between treatments. Here, at Figure 4, we can see a distinct formation, with stress of 0.2013, of two separate groups not sharing even a point.

Figure 4 – NMDS of insect community in both treatments showing formation of two distinct groups with Stress of 0.2013. Perdões, Brazil, 2018.



As NMDS show group formation, ANOSIM shows this difference in numbers, when the result is significant ( $< 0.05$ ) the groups formed are considered different (Clarke 1993) and, as possible to observe the significance value for the difference of both treatments was 0.0268 (Table 3).

Table 3 – ANOSIM for both treatments, pairwise comparison with global  $R = 0.3315$ , significance level = 0.0283 and 9999 permutations. Perdões, Brazil, 2018.

	Sun	Shadow
Sun	-	0.0268
Shadow	0.0268	-

The difference observed in NMDS and ANOSIM can be measured by the analyse SIMPER (Similarity Percentage), which is a simple method to assess which species are primarily responsible for the difference found between the sampled groups (Clarke 1993). Here we can observe that only eight species, of 20, contributed for more than 70% of that difference



and both treatments showed a difference of 76% in their overall composition. Another point to observe is that, this difference of 70% was all due to Braconidae species.

Table 4 – Similarity Percentage Analysis (SIMPER) between treatments with an average overall dissimilarity of 76.05% showing mean abundance of each specie in each treatment. Perdões, Brazil, 2018.

Taxon	Av. dissim	Contrib. %	Cumulative %	Mean Sun	Mean Shade
<b>Bra sp.3</b>	11,99	15,76	15,76	2,17	0,333
<b>Bra sp.1</b>	8,813	11,59	27,35	0,5	1,83
<b>Bra sp.24</b>	8,175	10,75	38,1	0,667	1,33
<b>Bra sp.5</b>	5,832	7,669	45,77	-	1
<b>Bra sp.25</b>	5,072	6,67	52,44	-	0,833
<b>Bra sp.6</b>	4,654	6,12	58,56	0,667	1
<b>Bra sp.15</b>	4,644	6,106	64,67	0,167	0,667
<b>Bra sp.20</b>	4,174	5,489	70,15	0,333	0,667
<b>Eul sp.7</b>	3,141	4,13	74,28	0,5	0,333
<b>Eul sp.1</b>	2,794	3,674	77,96	0,5	-
<b>Bra sp.4</b>	2,753	3,621	81,58	0,333	0,333
<b>Bra sp.2</b>	2,322	3,053	84,63	0,167	0,333
<b>Mym sp.12</b>	2,115	2,782	87,41	-	0,333
<b>Eul sp.4</b>	2,089	2,747	90,16	0,333	-
<b>Bra sp.7</b>	1,995	2,623	92,78	-	0,333
<b>Bra sp.22</b>	1,313	1,726	94,51	-	0,167
<b>Eul sp.3</b>	1,267	1,667	96,18	0,167	-
<b>Mym sp.3</b>	1,028	1,351	97,53	0,167	-
<b>Bra sp.11</b>	0,9671	1,272	98,8	0,167	-
<b>Eul sp.2</b>	0,9133	1,201	100	0,167	-

#### 4 DISCUSSION

The bootstrap is an estimator to have an idea if the sampling was enough or not, comparing the bootstrap value with the richness found, utilizing Coleman's rarefaction curve (Coleman 1981), we can have a efficiency value (Efron and Tibshirani 1993). When the curves tend to get close, the collected was representative (Figure 1).

Even though there wasn't too much insects reared, as compared with other works with coffee, this number is significative, as we worked with only three families, as we didn't have found any Bethylidae and, the bootstrap significance was high (89.16%) as shown in Figure 1, and looking at the distribution of insects, Sun coffee had a more equitable distribution of abundance when looking at all three families, but Shadow coffee had a more equitable distribution of Braconidae.

According to (Fisher 1992) the ANOVA test can be done when treatments don't interfere with each other, in other words, each treatment should be isolated from others. As our treatments were apart from each other by more than 100 meters and, according to Gomez (2007) the effect of yellow pan traps is no more than 50 meters, we can consider that there is no interference or, significative interference among treatments. By this, we can submit the data to an ANOVA test, that we selected Scott-Knott test at 5% significance (Table 2).

The major abundance and diversity of insects in shade-coffee was expected, as the shade plants confer alternative hosts, shelter and food to parasitoids (Root 1973; Andow 1991; Landis and Menalled 1996) and *Vachellia farnesiana* has a great flowering period and abundant flowering, meaning that there is plenty of food for parasitoids, also, shade provide a more suitable environment for parasitoids to live (Soto-Pinto et al. 2000) as also observed by Perfecto et al. (2003) and Borkhataria et al. (2012).

Richness was not significant, but as other works with coffee have shown, coffee by itself can harbour a great number of species as showed by Perioto et al. (2002), Gomez (2007), Fernandes (2013), Ferreira et al. (2013) and Tomazella (2016) so the addition of *V. farnesiana* wasn't enough to increase this richness.

Braconidae being the more collected family is very important, as there are many leaf miner and also borer beetle parasitoids within this family, as *Heterospilus coffeicola* (Hymenoptera: Braconidae) (Schimideknecht, 1924), (Hanson and Gauld 2006), *Orgilus niger* (Penteado-Dias, 1999), *Stiropius letifer* (Mann, 1872), *Eubazus punctatus* (Ratzeburg, 1852) (REIS et al. 2002) meaning that this number of invidious is very important and significative and the Shaded Coffee harboured a huge abundance of this family, and almost all morphospecies reared, excluding one, the Bra sp.11, but with no much importance, as only one specimen was found.

By the other hand, Eulophidae, also a very important parasitoid family, having exemplars as *Phymastichus coffea* (Hymenoptera: Eulophidae) (LaSalle, 1990), a borer beetle parasitoid and *Closterocerus coffeellae* (Ihering, 1914), *Proacrias coffeae* (Ihering, 1914), *Horismenus aeneicollis* (Ashmead, 1904), leaf miner's parasitoids (REIS et al. 2002) except for one morphospecies, were all found on Sun Coffee, going against hand with almost all works with shaded coffee.

There is a difference in the family's community between Shaded and Sun Coffee, as possible to see in Figure 3 and Table 3, and the main difference on those was because of eight

Braconidae morphospecies, that contribute to 70% of the difference most of all with higher mean at shade coffee.

With this, we can conclude that, the utilization of shade in coffee crop, enhance the Abundance and Diversity of important parasitoid families, such as Braconidae and Mymaridae, but for Eulophidae, the treatment without shadow was better. Those results are contrasting with other founds by some authors, but, this represents that we have to take a close look to the environment and the landscape to try to find a more robust result.

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## **ARTICLE 3 – DOES DIVERSIFICATION HAVE THE SAME EFFECT OF NATIVE VEGETATION SURROUNDING COFFEE PLANTATION ON PARASITOIDE FAMILIES?**

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Prepared according model of Neotropical Entomology

### **ABSTRACT**

In Brazil, coffee crop is mainly cultivated under monoculture conventional system, but this system has its drawbacks, as the simplification of the agriculture lead to a loss of many ecological services such as pest control, an ecological service very important to maintain many insects below pest status and also maintain pest at population levels that don't cause harm to the crop. Fortunately, there are at least two ways to mitigate this situation a) improve the diversification of the crop or, b) benefits from natural native vegetation, such as vegetation corridors or vegetation fragments. Both are proved to have beneficial increments to the crop by enhancing diversity of beneficial insects, as parasitoids. Many works showed an improvement in abundance, richness, and diversity of these insects, but none have shown the influence on specifically parasitoids of coffee crop pests. Our work had the aim to investigate the differences of a diversified coffee, a monoculture coffee, and the native surrounding vegetation on four important coffee parasitoid families. The work was conducted at Fazenda da Lagoa Km 642 of BR 381, municipality of Santo Antônio do Amparo, Minas Gerais, Brazil, in a coffee plantation (*Coffea arabica L.*) cultivar Catuaí 99, six years old. Were utilized plots of intercropped coffee with different tree species to compose our Diversified treatment, the native vegetation at coffee crop boarder that composed our Native treatment and a Monoculture coffee for comparison. The insects were collected utilizing yellow pan traps that stayed on the field for a period of 48 hours in all habitats. The insects were kept in alcohol 70% and identified to morphospecies. We found 157 specimens, being 29 morphospecies of Braconidae, 8 morphospecies of Eulophidae and 12 morphospecies of Mymaridae. Also, was possible to observe the formation of three distinct groups being the more abundant, rich and diverse the Diversified treatment.

**KEYWORDS:** Braconidae; Eulophidae, Mymaridae, Valos, Vegetation Corridors

## **1 INTRODUCTION**

The ecosystem can provide a huge variety of services and goods that we, as humans, can and should benefit from. Those services are normally set aside due to common sense of what came from nature is “free” and there are not a value associated with it (de Groot 1987). But, when we take those services in account, we can see it's potential, as showed by Losey and Vaughan, (2006) the service of pest control conducted by natural enemies contributes to more

than 4 billions of dollars in the United States and maintain around 65% of the potential pests under control.

Conservation biological control is based on the utilization of those service and focus on maintaining natural enemies in the cultivation area using attractive plants, making a complex spatial arrangement, and maintaining native border vegetation (Barbosa 1998). These plants will provide, among other things, shelter, and alternative food for natural enemies. This vegetal diversification provides an increase in the number of natural enemies in the area, allowing them to act in the regulation of pest (Root 1973; Barbosa 1998; Altieri 1999; Altieri et al. 2003). This pest regulation can contribute to an mortality of up to 80% of the leafminer insect (Pereira et al. 2007).

With the increase in the diversity of the environment, natural enemies are helped in several ways, especially through the provision of alternative food for adults, such as nectar, pollen and sugary substances; the availability of shelter and adequate microclimate, and the presence of prey and alternative hosts for natural enemies (Landis et al. 2000; Altieri et al. 2003). It is know that the diversification in coffee system promotes an increase in the diversity of ants (Melo et al. 2007; Moreno et al. 2009; De la Mora et al. 2015), birds (Gleffe et al. 2006), mammals (Daily et al. 2003) and parasitoids (Perfecto et al. 2003; Pak et al. 2015).

There is many ways to improve the conservation biological as for example, maintain the surrounding vegetation, that can enhance natural enemies abundance and diversity (Dainese et al. 2019). There are many ways to utilize the surrounding vegetation, as native fragments near the crop, vegetation corridors and, specifically in southern Minas, Valos, all these can increase the diversity of natural enemies and the movement between crop fields (Altieri et al. 2003). Valos are typical landscape structure found in all south of Minas Gerais state. These structures are composed of a channel sculped on the ground to separate farms on the past, that are now colonized by a great variety of trees and are now treated as vegetation corridors (Castro 2004). These valos can contribute with the parasitoid fauna and near it, the presence of parasitoids is increased (Gomez 2007). These valos are very important corridors and act as safety routes for mammals (Mesquita and Passamani 2012) and also, nesting grounds for many species of birds (Corrêa and Moura 2009).

Another way is the diversification of the crop with many kinds of plants, as is widely known, the diversification improve the diversity of arthropods and other organisms inside the crop an contribute to pest control and other services (Andow 1991; Altieri et al. 2003; Tschamtkke et al. 2007; Altieri and Rogé 2009; Nicholls and Altieri 2013). An alternative to

diversify the crop is the utilization of trees that, beyond the benefits of diversification by its own, it contributes to the enhancement of organic matter, nutrients, soil conservation and alternative food for natural enemies (Guharay 2001), promotes an increase in the diversity of insects, birds and other organisms that help in the control of possible pests (Perfecto et al. 1996, 2003; Soto-Pinto et al. 2000; Borkhataria et al. 2012) and allows the farm to sell the coffee out of commodity prices (Khatounian 2001).

Other thing is, as there are natural enemies and insects the occur at the crop but don't, or are found less abundant, than at native vegetation (Derocles et al. 2014) and its known that coffee can harbour by its own a great variety of parasitoids families (Perioto et al. 2004; Fernandes 2013; Ferreira et al. 2013; Tomazella 2016), and that's important, but, what happens with the actual parasitoids of the main pests of coffee that are mainly the families Braconidae, Eulophidae (Reis and Souza 2002; Reis et al. 2002; Hanson and Gauld 2006), Bethylidae (Ferreira 1980; Waller et al. 2007) and possible Mymaridae (Vega and Kirkl 1999).

With this in mind, this world had the goal to evaluate the differences in the abundance, diversity and richness of parasitoids of the families Braconidae, Eulophidae, Mymaridae and Bethylidae between a coffee monoculture, diversified coffee, native vegetation fragment and a Valo in southern Minas Gerais-Brazil.

## **2 MATERIAL AND METHODS**

### **2.1 Sampling Area**

The work was conducted at Fazenda da Lagoa Km 642 of BR 381, municipality of Santo Antônio do Amparo, Minas Gerais, Brazil (20°91'S/44°85'W/1100m) in a coffee plantation (*Coffea arabica L.*) cultivar Catuaí 99, six years old, landed by the farm to be conducted the experiment. The coffee crop area is a landscape mosaic with vegetation corridors, native fragments, coffee monoculture and diversified coffee.

The coffee, grown at 3.40m x 0.65m spacing, was conducted in the conventional cultivation system with total control of spontaneous plants, leaving the crop always clean. The cultural treatments, as well as the harvest, were carried out mechanically. The control of pests and diseases was carried out following the premises of the IPM, in which a monthly survey of the occurrence of pests was carried out and, after evaluation, the appropriate decision was made. If control was necessary, they performed insecticide applications.



Our treatments (Figure 1) were composed of Monoculture (Coffee crop cultivated under conventional monoculture), Diversified (Coffee crop intercropped with Avocado(*Persea Americana* Mill.), Mangium(*Acacia mangium* Wild), Red Cedar(*Toona ciliata* M. Roem.), Macadamia(*Macadamia tetraphylla* L.A.S.Johnson), Teak(*Tectona grandis* Linn.) and African mahogany (*Khaya ivorensis* A. Chev.) and Native (Vegetation fragment and Valo surrounding coffee crop).

The companion species were cultivated in the same period, therefore having the same age as the coffee plantation. However, due to physiological characteristics, they differed in size and phenological period.

Figure 1 - Aerial view of the experiment area with delimitation of each treatment showing yellow dots representing the yellow pan traps utilized. Santo Antônio do Amparo, Brazil, 2018.



## 2.2 Sampling

Our samples were conducted from June/2016 to March 2018, totalling eight collects. Was utilized yellow pan traps with 20 cm in diameter and 5 cm depth suspended 50 cm from the floor affixed to a bamboo stick. The traps were left in the fields for 48 hours in each collect containing a 10% saline solution of NaCl and neutral detergent.

For the Monoculture, each sample consisted of six spots inside the crop with the traps allocated in the line of plantation. Each spot was at least 50 meters apart of each other. For the Diversified treatment, were utilized six spots. Each spot was chosen in the middle of the plot containing a tree species placed in the line of plantation near to the tree species. For the Native treatment, were taken six spots, three from the Valo and three from the native fragment. We opted for this arrangement due the lack of difference between each tree utilized for intercrop the coffee relating to the parasitoid community, as possible to see in the Article 1 of this thesis.

## 2.3 Data Analyses

The insects collected were kept in alcohol 70 % and identified up to the family level according to didactic material by Goulet and Huber (1993) and separated into morphospecies and subsequently identified up to genus and species.

For data evaluation, the following analyses were made using the software R Studio (RSTUDIO TEAM, 2016), PAST® (Hammer, Harper, & Ryan, 2001), and Microsoft Excel 365 and the data were transformed to  $\sqrt{\text{data} + 0.5}$ , when necessary.

1) Rarefaction curves of species collected according to Coleman, (1981), which allow us to conclude whether the samples were regular and sufficient to collect, potentially, all species that occur in the culture.

2) Bootstrap wealth estimator (or one that is more appropriate), which uses data from all species collected to estimate total wealth, not being restricted to rare species.

3) Species wealth (S), which is the total number of species and morphospecies collected.

4) Abundance index, according to Lamshead, Platt, & Shaw, (1983), calculated from the means of each species per sample.

5) H' diversity index, according to Shannon & Weaver, (1949), which takes into account the quantitative uniformity of each species in relation to the others.

6) Similarity index, calculated by Cluster analysis, according to Pielou, (1984), which indicates how similar two substrates can be in relation to the species found.

7) NMDS analysis (non-metric multidimensional scaling) (Hennebert and Lees 1991), which graphically shows the difference in similarity between treatments.

8) Similarity analysis (ANOSIM) (Clarke 1993) consists of a non-parametric test to indicate a significant difference between two or more groups based on any measure of distance. In the case of this work, the Bray-Curtis measure will be used, as it is a robust similarity measure to work with abundance data (Bray and Curtis 1957).

9) Analyse SIMPER (Similarity Percentage), which is a simple method to assess which species are primarily responsible for the difference found between the sampled groups (Clarke 1993).

For the data to be available for ANOVA test the treatments should be separated and isolated from each other with no direct interference upon them from each other (Fisher 1936). When utilizing yellow pan traps to collect, its maximum efficiency radius can be considered to be 50 meters (Gomez 2007), as our treatments are apart from each other by more than this, we can use ANOVA on them. As so, we applied ANOVA test on Abundance, Richness and Diversity and found the following results (Figure 2).

### 3 RESULTS

To have an idea if the sampling was enough or not, we can utilize the Bootstrap estimator, for example, that predicts the possible number of species that can be reared from a local (Efron and Tibshirani 1993) and comparing this with the amount found, we can have an efficiency value (Coleman 1981). In our work we found 49 species and the bootstrap estimator predicted 54.81 species, that give us an efficiency of 89.39%.

As results of our collects we found a total of 157 specimens belonging to three families of parasitoids, being 29 morphospecies of Braconidae, 8 morphospecies of Eulophidae and 12 morphospecies of Mymaridae (Table 1), no Bethylidae was found. In our work we found 49 species and the bootstrap estimator predicted 54.81 species, that give us an efficiency of 89.39%.

For Braconidae family was found 95 individuals and the treatments that it was most found were Diversified and Native, both with 38 individuals. At the monoculture was found only 19 individuals. For Eulophidae, that had an abundance value of 23, the Native treatment was the higher, with 11 specimens, follow by Diversified with seven specimens and

Monoculture with five specimens. For Mymaridae, with 39 individuals found, the diversified that was the higher, with 23 individuals, followed by Diversified with 12 and the less abundant was monoculture with only four individuals.

At the Diversified treatment was found 68 individuals, being this 43.31% of all insects collected. And from these, 55.9% was from Braconidae family. For the Native treatment, were it was found 61 individuals, which was 38.85% of all insects, and 62.3% was Braconidae. And for Monoculture, the abundance was 28, being 17.83% of all insects, and also, Braconidae was the highest with 67.9% (Figure 2).

Table 1 – Abundance (X) and frequency (%) of taxons sampled from Diversified coffee, Monoculture coffee and Native Surrounding vegetation corresponding to the families Bra = Braconidae; Eul = Eulophidae and Mym = Mymaridae. Santo Antonio do Amparo, 2018.

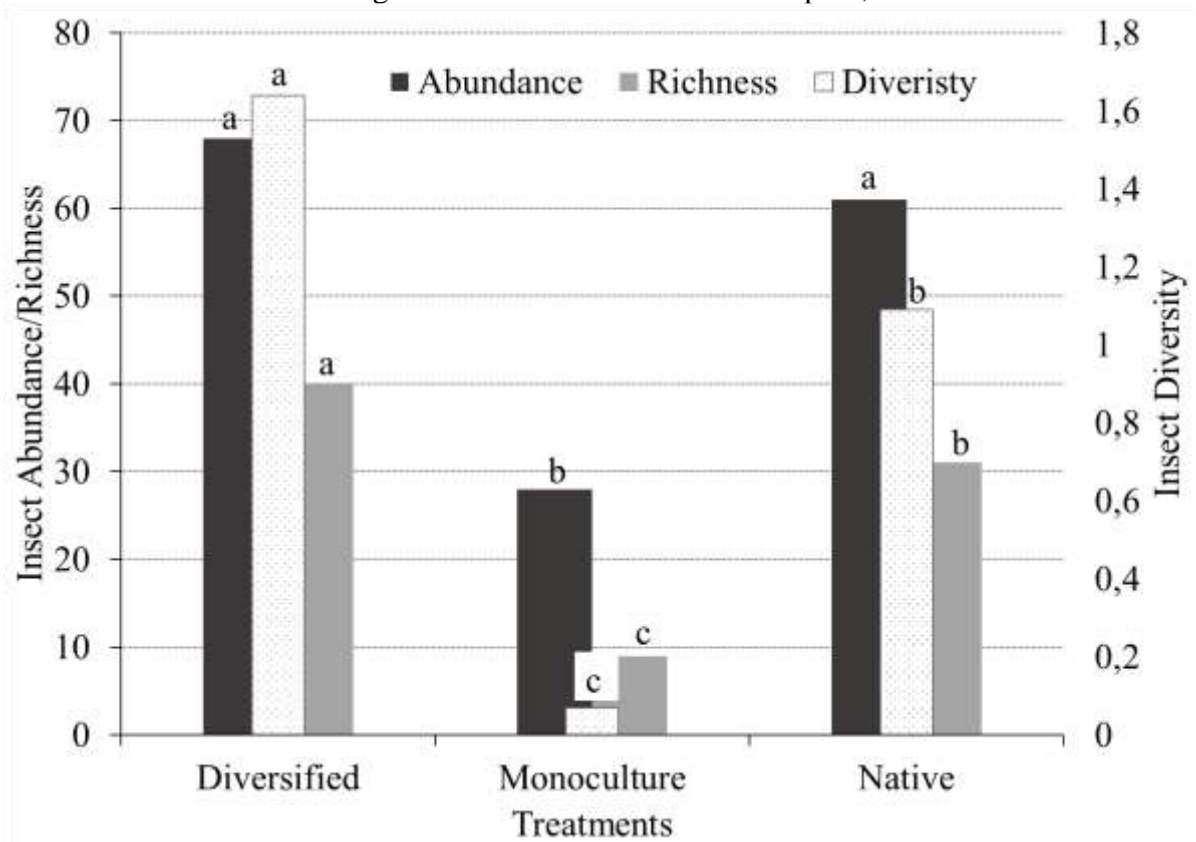
Family/ Morphospecies	System					
	Diversified	%	Monoculture	%	Native	%
<b>Braconidae</b>						
1. Bra sp.1	9	13.2	6	21.4	5	8.2
2. Bra sp.2	2	2.9	-	-	-	-
3. Bra sp.3	3	4.4	5	17.9	4	6.6
4. Bra sp.4	1	1.5	-	-	5	8.2
5. Bra sp.6	1	1.5	-	-	2	3.3
6. Bra sp.8	-	-	-	-	1	1.6
7. Bra sp.9	1	1.5	-	-	3	4.9
8. Bra sp.10	-	-	-	-	1	1.6
9. Bra sp.11	1	1.5	5	17.9	3	4.9
10. Bra sp.12	3	4.4	-	-	1	1.6
11. Bra sp.13	1	1.5	-	-	1	1.6
Family/ Morphospecies	System					
Diversified	%	Monoculture	%	Native	%	
12. Bra sp.14	2	2.9	-	-	-	-
13. Bra sp.15	3	4.4	3	10.7	5	8.2
14. Bra sp.16	1	1.5	-	-	-	-
15. Bra sp.17	1	1.5	-	-	-	-
16. Bra sp.18	1	1.5	-	-	-	-
17. Bra sp.19	1	1.5	-	-	-	-
18. Bra sp.20	1	1.5	-	-	-	-
19. Bra sp.21	-	-	-	-	1	1.6
20. Bra sp.22	1	1.5	-	-	1	1.6
21. Bra sp.23	-	-	-	-	1	1.6
22. Bra sp.25	1	1.5	-	-	-	-
23. Bra sp.26	1	1.5	-	-	-	-
24. Bra sp.27	1	1.5	-	-	-	-

Table 1 - Continuing

25. Bra sp.28	-	-	-	-	2	3.3
26. Bra sp.29	1	1.5	-	-	-	-
27. Bra sp.30	1	1.5	-	-	-	-
28. Bra sp.31	-	-	-	-	1	1.6
29. Bra sp.32	-	-	-	-	1	1.6
<b>Subtotal</b>	<b>38</b>	<b>55.9</b>	<b>19</b>	<b>67.9</b>	<b>38</b>	<b>62.3</b>
<b>Eulophidae</b>						
30. Eul sp.1	-	-	-	-	1	1.6
31. Eul sp.2	2	2.9	-	-	3	4.9
32. Eul sp.3	1	1.5	-	-	1	1.6
33. Eul sp.4	1	1.5	3	10.7	2	3.3
34. Eul sp.5	1	1.5	2	7.1	-	-
35. Eul sp.6	1	1.5	-	-	-	-
36. Eul sp.7	-	-	-	-	1	1.6
37. Eul sp.8	1	1.5	-	-	3	4.9
<b>Subtotal</b>	<b>7</b>	<b>10.3</b>	<b>5</b>	<b>17.9</b>	<b>11</b>	<b>18</b>
<b>Mymaridae</b>						
38. Mym sp.1	4	5.9	-	-	2	3.3
39. Mym sp.2	2	2.9	-	-	1	1.6
40. Mym sp.3	1	1.5	2	7.1	1	1.6
41. Mym sp.4	2	2.9	-	-	1	1.6
42. Mym sp.5	2	2.9	1	3.6	3	4.9
43. Mym sp.6	1	1.5	-	-	-	-
44. Mym sp.7	2	2.9	-	-	-	-
45. Mym sp.8	1	1.5	-	-	-	-
46. Mym sp.9	3	4.4	1	3.6	2	3.3
47. Mym sp.10	3	4.4	-	-	1	1.6
48. Mym sp.11	1	1.5	-	-	1	1.6
49. Mym sp.12	1	1.5	-	-	-	-
<b>Subtotal</b>	<b>23</b>	<b>33.8</b>	<b>4</b>	<b>14.3</b>	<b>12</b>	<b>19.7</b>
<b>Total</b>	<b>68</b>	<b>43.31</b>	<b>28</b>	<b>17.83</b>	<b>61</b>	<b>38.85</b>

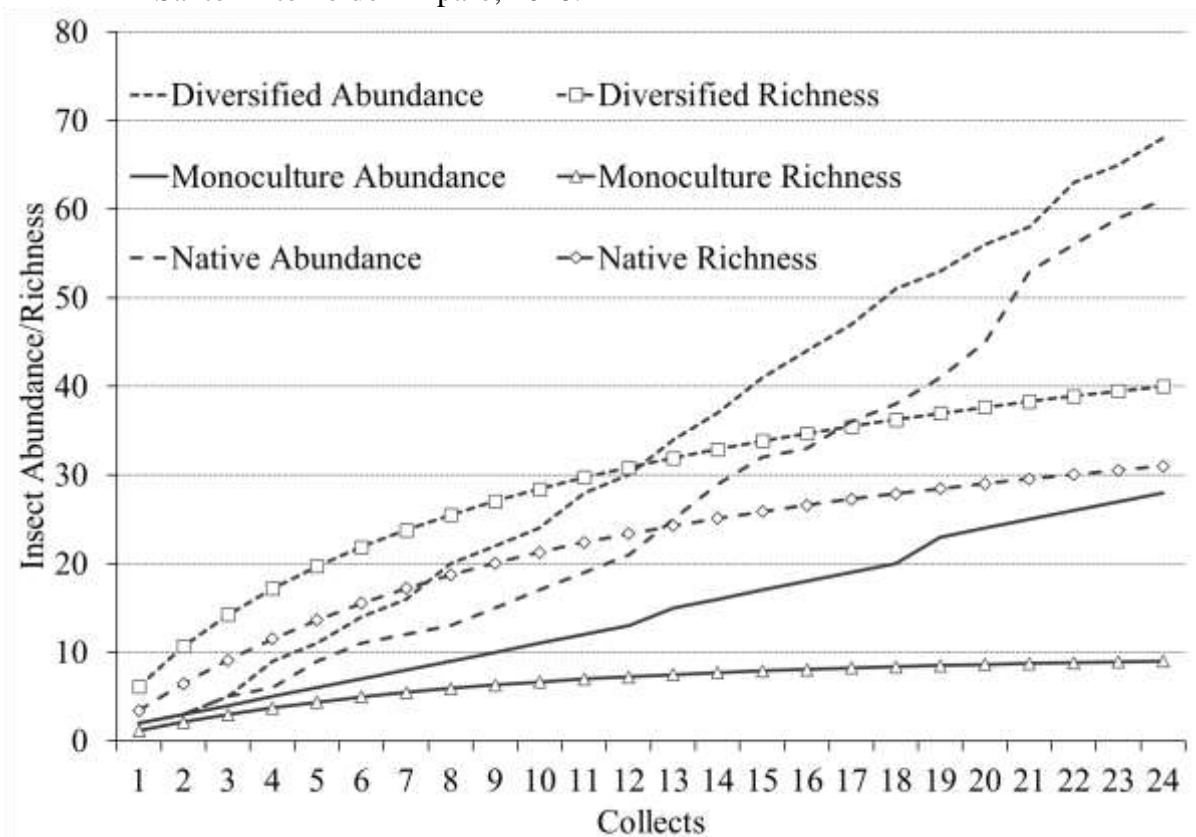
Looking at the insect Richness at the treatments, from 49 species sampled in overall, 40 of them were found on the Diversified treatment, 31 at Native treatment and only 9 at Monoculture treatment, as represented in Figure 2. The diversity  $H'$  was higher at Diversified treatment with a value of 1.64, followed by Native treatment with 1.09 and the lowest was Monoculture with only 0.07.

Figure 2 – Diversity index of each treatment showing small letters on top of it representing the statistical difference. Different small letters represent statistical difference by Scott-Knott test at 5% significance. Santo Antonio do Amparo, 2018.



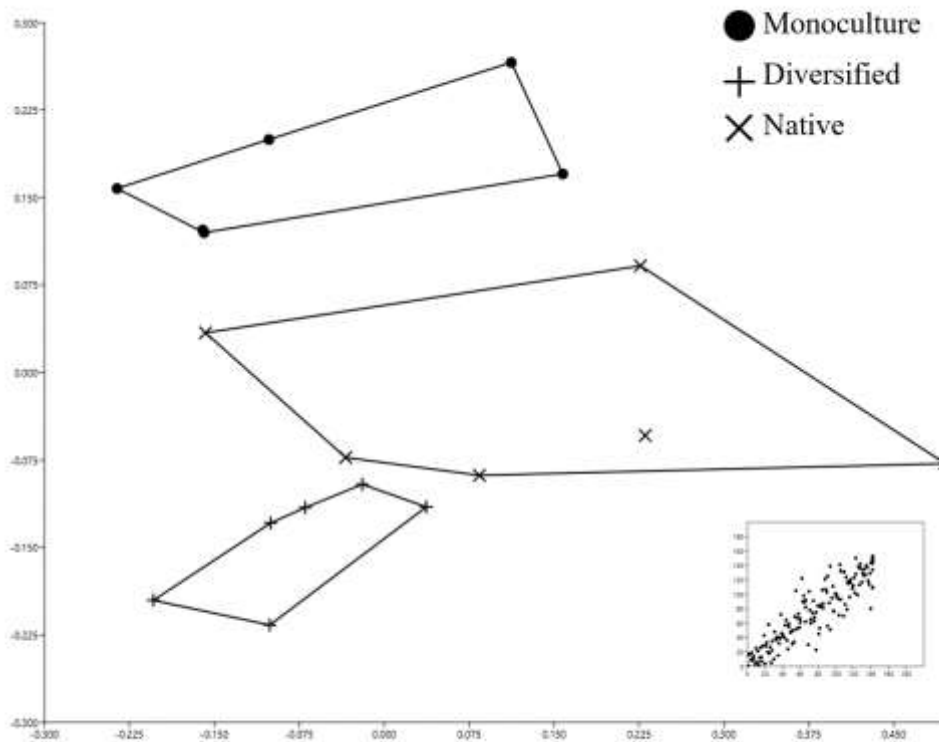
A good way visualizes the abundance and richness along the sampling period is in a graphic, and here (Figure 3) we can see that Abundance at Native and Diversified, from the beginning were higher than Monoculture, and had a constant increment over time. For Richness, if possible, to observe a pattern since from the first collect, with Diversified being the highest followed by Native and then Monoculture, that practically had no increase in its richness from collect 18 to the end.

Figure 3 – Graphic representation of accumulated abundance and richness in all treatments. Santo Antonio do Amparo, 2018.



With NMDS we can find if the treatment were different based on the insect community, and, here, we found three different and separated groups (Figure 4) with the stress run of 0.21.

Figure 4 – NMDS of insect community in all treatments showing the distinct formation of three separated groups with Stress of 0.21. Santo Antonio do Amparo, 2018.



We run the ANOSIM (Table 3) test to confirm the group formation, and when the result are significant ( $< 0.05$ ) the treatments can be considered different (Clarke 1993) and this result confirm the grouping formed by the NMDS. Also, the SIMPER test shows which insect contributed to this difference and here we illustrated the insects that contribute up to 70% of the total difference (Table 4).

Table 3 – ANOSIM of treatments with 9999 permutations, R global= 0.3831, with significance level  $p = 0.0001$ . Santo Antonio do Amparo, 2018.

	<b>Diversified</b>	<b>Monoculture</b>	<b>Native</b>
<b>Diversified</b>	-	<i>0,0022</i>	<i>0,0049</i>
<b>Monoculture</b>	-	-	<i>0,0455</i>
<b>Native</b>	-	-	-

After looking at NMDS and ANOSIM, we found that there was a difference of 78.2% between all treatments and half (20) of the total species (40) found, contributed to 71.2% of this difference. No species alone contributed with more the 7% of the difference.



Table 4 – Similarity Percentage Analysis (SIMPER) between treatments with an average overall dissimilarity of 78.2% showing mean abundance of each specie in each treatment. Santo Antonio do Amparo, 2018.

<b>Taxon</b>	<b>Av. dissim</b>	<b>Contrib. %</b>	<b>Cumulative %</b>	<b>Mean diver</b>	<b>Mean mono</b>	<b>Mean native</b>
<b>Bra sp.3</b>	5.0	6.4	6.4	1	0.8	0.6
<b>Bra sp.1</b>	4.6	5.9	12.4	1.6	1	0.8
<b>Bra sp.15</b>	3.9	5.0	17.4	0.8	0.5	0.8
<b>Bra sp.4</b>	3.5	4.4	21.9	0.5	-	1
<b>Eul sp.2</b>	3.3	4.2	26.2	0.6	-	0.6
<b>Bra sp.11</b>	3.3	4.2	30.4	0.1	0.8	0.5
<b>Mym sp.1</b>	3.1	4.0	34.4	1	-	0.5
<b>Mym sp.5</b>	3.0	3.9	38.3	0.6	0.1	0.6
<b>Eul sp.4</b>	3.0	3.8	42.2	0.1	0.5	0.5
<b>Mym sp.10</b>	3.0	3.8	46.0	1	-	0.1
<b>Mym sp.9</b>	2.6	3.3	49.4	0.8	0.1	0.3
<b>Bra sp.12</b>	2.5	3.3	52.7	0.8	-	0.1
<b>Eul sp.8</b>	2.2	2.9	55.7	0.1	-	0.6
<b>Mym sp.4</b>	1.8	2.3	58.0	0.5	-	0.1
<b>Bra sp.9</b>	1.8	2.3	60.4	0.1	-	0.5
<b>Bra sp.6</b>	1.8	2.3	62.7	0.3	-	0.3
<b>Eul sp.5</b>	1.7	2.2	64.9	0.3	0.3	-
<b>Bra sp.14</b>	1.7	2.2	67.2	0.6	-	-
<b>Mym sp.3</b>	1.6	2.1	69.3	0.1	0.3	0.1
<b>Eul sp.3</b>	1.5	1.9	71.2	0.1	-	0.3

#### 4 DISCUSSION

It is well known that the diversification and the surrounding vegetation improve the diversity of parasitoids at coffee crops, as possible to see at works conducted by Perioto et al. (2002); Perioto et al. (2004); Palma-Santos and Pérez-Maluf (2010); Fernandes (2013); Ferreira et al. (2013); Lara and Perioto (2014); Tomazella (2016) but, none focusing only on parasitoid families that have lot of important parasitoids to coffee pests as families Braconidae, Eulophidae, Mymaridae and Bethyridae, that shows a great number of individuals that parasitize coffee pests (Goulet and Huber 1993; Vega et al. 1999; Reis and Souza 2002; Reis et al. 2002; Hanson and Gauld 2006; Chapman et al. 2008).

As the works cited above, we found similar results, but what was different was that we found a greater abundance, richness, and diversity at the Diversified system than in the native system. First, related to insect abundance we found 68 insects in Diversified treatment, 61 in Native treatment and only 28 in Monoculture treatment. This result are well satisfactory as it fall right under diversification theories that, a) Resource concentration theory, that predict that herbivores have more trouble to find and colonize the host plant due to great abundance of smells and other stimulus (Root 1973; Andow 1991) and b) Natural Enemies Hypothesis, that predict that the abundance of natural enemies at diversified environments are greater than less diversified due to the greater availability of resources and shelter (Andow 1991; Landis et al. 2000).

Now, looking at insect richness and diversity, that at our work, it was clearly different for all three treatment (Figure 2), the diversified treatment, again, was higher, but now, than the both others. It is well known that native and natural vegetation harbour a greater richness than agroecosystems and the utilization of plants to diversify the culture is a practical to improve those systems (Altieri 1999; Altieri, M y Nicholls 2000; Altieri et al. 2003; Letourneau et al. 2009), but, here, we only looking at four particular families of insects that have many parasitoids of coffee pests, so, as at native vegetation no host can be found, the diversified treatment take the stage as enhancing the agricultural matrix, the quality of the resources as food, nesting resources and shelter are improved (Landis et al. 2000; Vandermeer et al. 2010), explaining why the Diversified treatment showed higher values than Native.

We had the formation of three distinct groups, as possible to see at Figure 3 and attest at Table 3, and the explanation to this formation is quite simple, there are insects that occur at crop and don't occur at native vegetation (Derocles et al. 2014) making it more abundant at the monoculture and almost not found at the Native treatment, as the environment are less suitable for those insects. The opposite is also true, as there are many insect species that are not found at the monoculture crop due to the lack of its food (Altieri and Letourneau 1982; Altieri and Rogé 2009). So, with the addition of plants to diversify the crop, as trees (our Diversified treatment) the resources found are the same as the monoculture, plus the resources offered by the trees, and thus implying in a greater abundance, richness and diversity than the monoculture and native treatment, as the native treatment lack the resources provided by the coffee crop (Altieri and Rogé 2009; Dainese et al. 2019).

So on, we can look at which species contribute to this difference and we found that 20 species contributed to more than 70% of this difference (Table 4). Taking a close look, the

individual contribution to this difference was quite similar, being the highest Bra sp.3 that contribute with 6.4% of it and it was found more frequently at diversified treatment.

With our work results, we can conclude that the diversification of the coffee crop with tree species, contributes to an enhancement of the Abundance, Richness and Diversity of the three of the four parasitoid families. With this, do not exclude the importance of the native fragments and corridors, as many other works shows its importance in many aspects. And at last, with this work, we conclude that the monoculture coffee, taking in account the parasitoid abundance, richness, and diversity, are far worse than diversified coffee.

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