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Diversity of Hymenopteran Parasitoids in Coffee Plantations under Agroecological Transition and Its Impact on Coffee Leaf Miner (*Leucoptera coffeella*) Infestations

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Abstract: The biodiversity of natural competitors is vital to key ecosystem services and agroecosystems' benefits to society. The abundance and richness of hymenopteran parasitoid communities, and subsequently their services, are dependent on the variety of habitats in the different agroecological landscapes. Here, we monitored the fluctuation of predatory wasps and hymenopteran parasitoid populations and their impacts on coffee leaf miner infestations under different coffee plantation landscapes. Thus, 24 sampling plots were arranged in four cultivation systems: conventional (CONV), in transition to organic shaded (T.OSH), in transition to organic full-sun (T.OFS), and without pesticide (T.WOP). In each plot, leaves with intact mines were collected randomly once a month over a period of 23 months. Parasitoid species, coffee leaf miner infestation, predation, and parasitism were assessed based on the emerged parasitoids and wasps' activity signs in the mines. The data on parasitoids revealed the presence of 621 hymenopteran parasitoids, of which, 420 were Braconids and 201 were Eulophids. Overall, the abundance of braconid specimens (67.6%) was greater, but the species richness was higher in Eulophids. The highest species richness of *L. coffeella* parasitoids was in T.OSH and T.OFS. Furthermore, in the coffee plants evaluated, the coffee leaf miner population is well established and does not cause economic damage, as the spontaneous action of predatory wasps and parasitoids, in addition to climatic factors, contributes to regulating these pest infestations. Of these parasitoids, the braconid *Orgilus niger* and *Stiropius reticulatus* were found to be promising and well-adapted control provider species.

Keywords: biological control; Braconidae; Eulophidae; agroecology; *Coffea arabica*

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1. Introduction

Brazil is the largest producer and exporter, the second largest consumer, and the world's largest source of sustainable coffee [1]. Minas Gerais is its highest-producing state, and the southern region of the state is the largest *Coffea arabica*-producing region [2]. According to [3] coffee, after oil, is the most valuable commodity in terms of total value (harvest per cup of coffee). Furthermore, due to the dependence of conventional producers on market demand and the growing demand for organic coffee [4,5], different coffee cultivation systems have been developed and can be found today in coffee production. Thus, the production of specialty coffees is becoming a competitive alternative to commodity coffee because of their monetary benefits over regular varieties, being worth up to six times more than commodity coffee [6].

The coffee plant can host a wide range of arthropods, and the coffee leaf miner *Leucoptera coffeella* (Guérin-Mèneville and Perrottet, 1842) (Lepidoptera: Lyonetiidae) is considered a key pest of the crop, causing large losses [7–10]. The impact of pest attacks on coffee plants causes losses of hundreds of millions of dollars every year [11]. In conventionally grown coffee, chemical pesticides are frequently used to control this and other pests, which can lead to disequilibrium in agroecosystems, since they kill not only insects and organisms considered pests but also the pests' natural enemies [12].

Successful experiences in agroecology have shown that the closer an agroecosystem is to the natural ecosystem, the more stable it tends to be [13–15]. Therefore, alternative production systems that consider this, unlike conventional systems, are potentially important for species conservation [12,16,17]. These agroecosystems are suggested to reduce the occurrence of pests and provide shelter for natural enemies due to the greater number of plant species found in these locations [18]. The shift from a conventionally managed to an alternative system is carried over a conversion period, which varies from 12 to 36 months according to the previous use and the current ecological situation of the agroecosystem. Studies on agroecological transitions have shown that the closer an agroecosystem is to the natural ecosystem, the closer it tends to reach equilibrium [19].

In this context, coffee agroecosystems have received growing attention for their apparent ability to protect biodiversity [17,20]. Moreover, natural enemies, especially predators and parasitoids, are important organisms that help regulate the populations of pest insects in coffee plantations [21–23]. A total of 28 species of parasitoids of coffee leaf miner are known worldwide, of which 13 occur in Brazil [7], especially wasps of the families Braconidae and Eulophidae [22]. Predatory wasps, with 11 species reported in Brazil, are efficient natural enemies of *L. coffeella* [7,24–28]. The mortality of leaf miners due to predatory wasps can vary between 10% and 70% [7,29,30]. The population dynamics of coffee pests depend on the coffee-producing region and the biotic and abiotic factors operating in the agroecosystem [31], and the population dynamics of the coffee leaf miner and its natural enemies have already been studied in conventional coffee cultivation systems. Yet, such dynamics in conventional crops under agroecological transition are poorly understood.

Here, we hypothesized that coffee systems under agroecological transition will present higher parasitoid diversity and abundance contributing to leaf miner control. Consequently, we recorded the coffee leaf miner population fluctuations in areas under conventional cultivation systems and agroecological transition, as well as the action of hymenopteran parasitoids in their regulation. The abundance, richness, diversity, population fluctuation, and parasitism rate of parasitoids of the families Braconidae and Eulophidae were also determined. This information may contribute to conservation and biological control recommendations for coffee production in southern Minas Gerais.

2. Materials and Methods

The surveys were conducted from February 2013 to December 2014 on 6.0 ha of reformed land areas in the Primero do Sul and Santo Dias settlements, located in the municipalities of Campo do Meio (21°07′19.09″ S 45°55′41.48″ W) and Guapé (20°49′57.15″ S 46°1′3.34″ W), both in Minas Gerais state (Brazil). According to the Koppen–Geiger classification, these cities have a humid subtropical climate (Cwa). These settlements are characterized by having the planting of coffee grown in the conventional system, that is, monoculture in full sun, as their main source of income. In addition to coffee, the production of grains such as maize, rice, and beans represents a part of their source of income and subsistence.

2.1. Establishment of the Agroecological Transition Units

A total of 24 plots of 2500 m^2 each with coffee plantations in production were demarcated in the municipality of Campo do Meio and the municipality of Guapé (8 plots). The plots were managed under four different production systems, with six replicates each: conventional coffee production (CONV), without pesticide (WOP), organic full-sun (OFS), and organic shaded (OSH) (Figure S1).

In the six plots under the conventional production system, all the management practices (including the use of chemical control and fertilization) already adopted by the producer were maintained and served as controls for the other systems. Fertilization was carried out with 900 kg/ha of formulated fertilizer (30% N; 20% K). During the experiment, fungicides with active ingredients pyraclostrobin and epoxiconazole were used, when necessary, in the rainy season, in addition to insecticides based on abamectin in December 2014, imidacloprid via soil once every two years, and herbicides based on glyphosate twice a year.

Under the three other production systems, agroecological transition-related practices were considered as follows. In the WOP production system, the fertilization practices already used by the producer were maintained. However, using chemical control for the management of pests, diseases, and spontaneous plants was replaced by using a Bordeaux mixture enriched with micronutrients.

In the OFS and OSH production systems, fertilization with synthetic chemical fertilizers was replaced by the use of organic fertilizers via soil and foliar application. The nitrogen source was legume meal (7.2% N) plus micronutrients at a dose of 5.0 tons per hectare or *Terra de Cultivo* ™ compost (1.68% N; 2.74% P2O5; 1% K2O) plus a bone meal at 5.0 ton/ha. The source of potassium was K-Mag (21% K; 21% S and 10% Mg) at 1200 kg/ha. The phosphorus source was Bayóvar natural phosphate rock (14% P and 32% Ca) at 335 kg/ha. Foliar fertilization was performed with *Calda Viçosa*, which provides nutrients to the plants and assists in the prevention and management of pests and diseases. The following nutrients were used: borax (15 to 30 kg/ha), tribasic copper sulfate (15 to 35 kg/ha), zinc sulfate (15 to 35 kg/ha), and manganese sulfate (15 to 35 kg/ha). Farmers were also encouraged to use organic compost and biofertilizers produced by them through the qualification course they received. Soil amendments were applied with calcitic limestone as the calcium source (54% Ca—PRNT 95%). Green fertilization was performed using a showy rattlebox (*Crotalaria spectabilis*) and pigeon pea (*Cajanus cajan*) between the rows of coffee plants following the technical recommendations for planting and management from the seed suppliers. In the organic shaded transition system, fruit species were added for afforestation.

2.2. Samples

Each month, we sampled the parasitoid hymenopteran populations and recorded the incidence, predation, and parasitism of the coffee leaf miner. All collected material was taken to the Entomology Laboratory of the IFSULDEMINAS Machado Campus and the Federal University of Lavras for evaluation.

2.3. Sampling of Hymenopteran Parasitoids Populations and Evaluation of the Parasitism Rate

Ten leaves with intact mines were randomly collected from each of the twenty-four plots by removing one leaf from the 3rd or 4th pair per plant from the middle or upper third as suggested by [32]. These leaves were individually placed in sealed plastic bags and kept in the laboratory under ambient conditions for 40 days, during which, the emerged parasitoids were collected. The parasitoids were counted and preserved in 70% alcohol for species' identification using different hymenopteran keys [33–39]. The parasitism rate of *L. coffeella* was calculated as % parasitism = number of parasitoids × 100/number of intact mines.

2.4. Sampling of the Incidence and Predation of the Coffee Leaf Miner

The occurrence of the coffee leaf miner was assessed as a function of the percentage of mined leaves. Ten sampling points were established in each cultivation system/area, and at each point, five leaves (one leaf/plant) from the 3rd or 4th pair were randomly removed from the middle third of the plant [32]. The percentage of infestation was calculated as % infestation = number of mined leaves/total number of leaves collected × 100. The tears in the lesions caused by the coffee leaf miner resulting from the action of wasps on the upper or lower surface of the leaves were quantified, and the predation rate was calculated as % predation = number of mined leaves/total number of predated leaves × 100. The average rainfall and temperature from January 2013 to December 2014 (historical averages) were obtained from the Regional Cooperative of Coffee Growers in Guaxupé (COOXUPÉ).

2.5. Faunal and Statistical Analysis

The species richness (S), Shannon–Wiener diversity (H′), and similarity (obtained by NMDS and Simper analysis, using the Bray–Curtis index of similarity) indices were calculated using Primer 6-Permanova+® [40] and EstimateS® [41] software. The species richness and abundance data were used to calculate the cumulative curve of individuals, the collector's curve (Coleman), the curve of unique and duplicate species, and the Chao 1 richness estimator, which is based on species richness and abundance.

The abundance, S, H′, parasitism and infestation rates, and the data on the main parasitoid taxa collected in the four systems were tested for homogeneity of variance. A Shapiro–Wilk normality test was conducted to test for parametric data. Generalized Linear Models (GLMs) were made using the 'stats' package, and models were tested for the best distribution for each variable (Poisson, Quasi-Poisson, Gaussian, or Gamma). Models of best fit were chosen based on AIC values and diagnostic residual plots. ANOVAs were then performed, and the results were reported as chi-square and *p*-values. Then, Tukey's Honest Significant Difference Test was used post-hoc to compare the treatments. The software RStudio© [42] was used for the calculations.

3. Results

The analysis of the rarefaction curves from the data of all collections showed that the Coleman rarefaction curve stabilized, especially because the curves of unique and duplicate species merged at the end of the sampling. These data indicated the depletion of new taxa in the study area as a whole. This was confirmed by the Chao 1 richness estimator which estimated eight parasitoid species (Figure S3).

Regarding the similarity between the systems, the cluster analysis indicated the greatest similarity (89.55%) between the system in transition to WOP (T.WOP) and the organic shaded system (T.OSH). The system in transition to organic full-sun (T.OFS) showed a similarity of 87.21% with the T.WOP/T.OSH pair. The lowest similarity (74.82%) was between the conventional (CONV) system and the systems in transition (Figure 1).

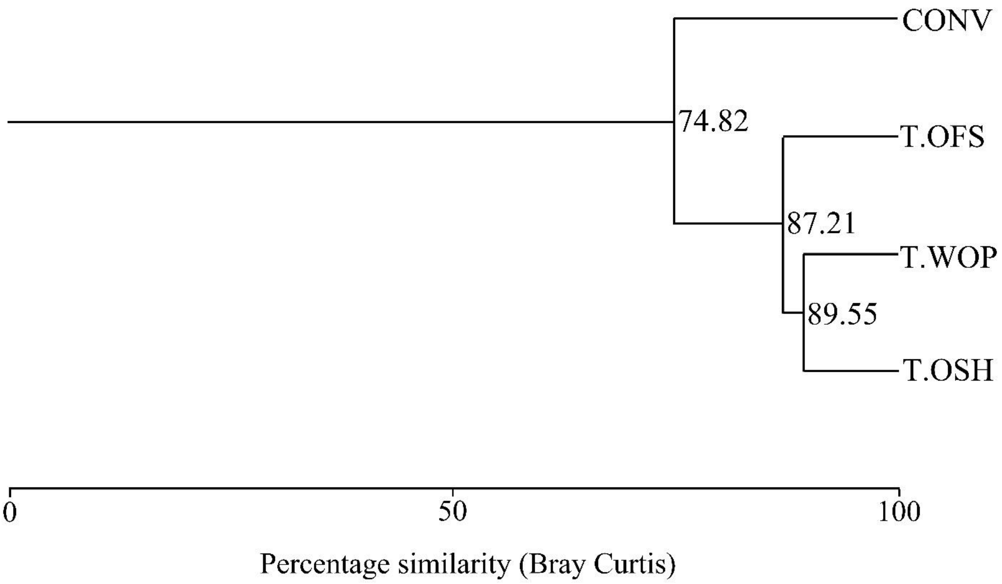

Figure 1. Diagram of the cluster analysis (Bray–Curtis index) indicating the similarities between the four coffee (*Coffea arabica*) production systems, namely, conventional (CONV) and transition to organic shaded (T.OSH), organic full-sun (T.OFS), and without pesticide (T.WOP). February 2013 to December 2014. Campo do Meio and Guapé, MG, Brazil.

The NMDS and ANOSIM analysis also showed no differences between the treatments according to their similarities (using the Bray–Curtis measure—Results of NMDS, and ANOSIM pairwise tests are presented in Figure S2 and Table S1).

From the Simper analysis of all treatments, it was observed that there is a general dissimilarity of 19.54%, and the species that generated the greatest difference were *O. niger*, *P. coffeae*, and *S. reticulatus*, the three together contributing 76.98%. Of the eight species collected, four contributed to 83.89% of the difference between treatments (Table 1).

Table 1. The percentage of similarity (SIMPER) of all treatments with an overall dissimilarity of 19.54%, showing average species contribution (C%), average dissimilarity (AC%), and average in each treatment for the species. The production system treatments were conventional (CONV), and transitioning to organic shaded (T.OSH), organic full-sun (T.OFS), and without pesticide (T.WOP). February 2013 to December 2014. Campo do Meio and Guapé, MG, Brazil.

Taxa	C%	AC %	CONV	T.OSH	T.OFS	T.WOP
O. niger	6.972	35.67	82	63	79	45
P. coffeae	4.423	22.63	46	38	31	21
S. reticulatus	3.651	18.68	35	26	26	45
C. striata	1.351	6.91	4	10	2	3
C. coffeellae	1.009	5.16	8	8	6	12
C. neotropicus	0.718	3.67	4	5	3	1
C. sp2	0.713	3.65	2	3	0	4
H. aeneicollis	0.707	3.62	4	2	3	0

3.1. Diversity Richness and Abundance of Hymenopteran Parasitoids

A total of 420 braconids and 201 eulophids were collected, belonging to three and five different taxa, respectively: *Orgilus niger* Penteado-Dias, 1999, *Stiropius reticulatus* Penteado-Dias, 1999, and *Centistidea striata* Penteado-Dias, 1999 (Braconidae); and *Proacrias coffeae* Ihering, 1913, *Closterocerus coffeellae* Ihering, 1914, *Horismenus aeneicollis* Ashmead, 1904, *Cirrospilus neotropicus* Diez and Fidalgo, 2004, and *Cirrospilus* sp. 2 (Eulophidae) (Table 2). There was a greater richness of Eulophidae than Braconidae, but the abundance of Braconidae was represented by 67.63% of the emerged specimens. In general, out of all systems analyzed, the most abundant species were *Orgilus niger*, *Stiropius reticulatus*, and *Proacrias coffeae*.

Table 2. Total abundance, relative frequency (RF%), richness, and Shannon diversity index (H') of the hymenopteran species associated with the coffee leaf miner *Leucoptera coffeella* in coffee plants (*Coffea arabica*) in Campo do Meio and Guapé, MG, Brazil.

Systems (n)	CONV (132)		T.OSH (132)		T.OFS (132)		T.WOP (132)					
Taxa	Total	RF%	Total	RF%	Total	RF%	Total	RF%	TOTAL	*F*	*D.F*	*P*
Centistidea striata	3	2.30	4	2.16	10	6.45	2	1.33	19	1.19	3	0.32
Orgilus niger	45	34.35	82	44.32	63	40.65	79	52.67	269	0.60	3	0.61
Stiropius reticulatus	45	34.35	35	18.92	26	16.77	26	17.33	132	0.54	3	0.65
Cirrospilus neotropicus	1	0.76	4	2.16	5	3.23	3	2.00	13	0.85	3	0.47
Cirrospilus sp. 2	4	3.05	2	1.08	3	1.94	0	0	9	1.26	3	0.29
Closterocerus coffeellae	12	9.16	8	4.32	8	5.16	6	4.00	34	0.32	3	0.80
Horismenus aeneicollis	0	0	4	2.16	2	1.29	3	2.00	9	1.26	3	0.29
Proacrias coffeae	21	16.03	46	24.88	38	24.51	31	20.67	136	0.55	3	0.65
ABUNDANCE	131	100.0	185	100.0	155	100.0	150	100.0	621	0.31	3	0.82
RELATIVE %	21.10%		29.79%		24.96%		24.15%		100.0			
RICHNESS	7		8		8		7		8	0.29	3	0.83
DIVERSITY (H')	1.477		1.455		1.583		1.31		-	0.70	3	0.55

GLM models adjusted to Gaussian distribution and tested with Tukey's test. Values between parentheses (n) next to the system names indicate the number of repetitions.

The abundance, S, and H′ of parasitoids were similar between the different systems. Both the parasitoids of the family Braconidae and those of Eulophidae tended to be more abundant in T.OSH. Regarding species richness, eight parasitoid taxa were collected from coffee plants in T.OSH and T.OFS and seven from plants in T.WOP. T.OFS showed the highest H′ index, but it was not significantly higher than the others.

The abundance, S, H′, of parasitoids collected in the four systems were tested for homogeneity of variance; Generalized Linear Models (GLMs) were used, and models were tested for the best distribution for each variable. The best model evaluated was the Gaussian, but the analysis of variance ANOVA showed no significant differences between the evaluated data.

3.2. Population Fluctuations of Coffee Leaf Miner Parasitoids

In the conventional system (Figure 2A), there was a greater abundance of the braconid *O. niger* in October and November 2013 and December 2014 and *S. reticulatus* in October 2013 and February 2014. The eulophid *P. coffeae* showed population peaks in April, July, and November 2013 and April and July 2014. In this system, from July to October 2013 and in February, March, and October 2014, the abundance of *S. reticulatus* was greater than that of *O. niger*; and from November 2013 to January 2014 and in December 2014, there was a significant increase in the abundance of this parasitoid and a decrease in *S. reticulatus*. In April and July 2013 and 2014, the parasitoid *P. coffeae* was more abundant than *O. niger* and *S. reticulatus.*

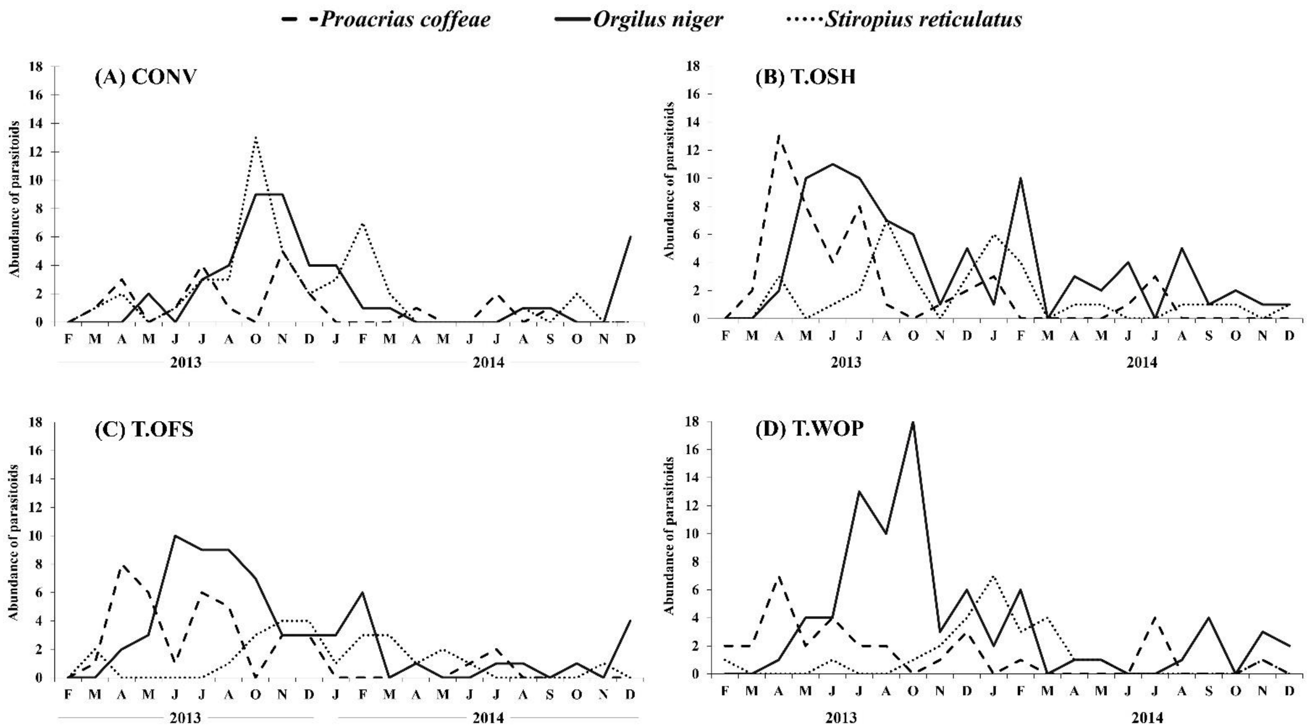

Figure 2. Population fluctuations of Braconidae and Eulophidae in the conventional system (CONV) and systems in transition to organic shaded (T.OSH), organic full-sun (T.OFS), and without pesticides (T.WOP). Campo do Meio and Guapé, MG, Brazil, February 2013 to December 2014.

In the T.OSH areas, there was a greater abundance of *O. niger* in June and July 2013 and most months of 2014. The parasitoid *P. coffeae* was most abundant in April 2013, with population peaks in April and June 2013 and January and July 2014, and the same was true of *S. reticulatus* in August 2013 and January 2014 (Figure 2B).

In the T.OFS areas, there was a greater abundance of *O. niger* from June to October 2013 and in February, October, and December 2014. The parasitoid *P. coffeae* was more abundant in April and May 2013, with population peaks in April and July 2013 and July 2014. The same was true of *S. reticulatus* in March, November, and December 2013 and February, March, and May 2014 (Figure 2C).

In the T.WOP areas, there was a greater abundance of *O. niger* parasitoids with population peaks in July, October, and December 2013 and in February, September, and November 2014, while *S. reticulatus* peaks were seen in January and March 2014. *P. coffeae* showed population peaks in April 2013 and July 2014. In all systems evaluated, a reduction in the population of the parasitoid *P. coffeae* was observed with the increase in the *O. niger* population, indicating possible competition between the two species. The same occurred with *S. reticulatus* and *O. niger* throughout the evaluated period (Figure 2D).

3.3. Infestation of Coffee Leaf Miner and Percentage of Predation and Parasitism

The percentages of coffee leaf miner infestation (%CLM), predation (%PRED), and parasitism (%PAR) were not significantly different between the systems studied (Table 3). In all systems studied, the %CLM did not vary considerably from 2013 to 2014 but never reached the control level of 30% of mined leaves. The %PAR was significantly higher in 2013 than in 2014 in each system (Table 4).

Table 3. The mean percentage of coffee leaf miner infestation (%CLM), predation (%PRED), and parasitism (%PAR) in conventional and three agroecological transitions systems of coffee plants *(Coffea arabica*) within two consecutive years (2013 and 2014).

System (n)	Year	% CLM (±SE) n.s	% PRED (±SE) n.s.	% PAR (±SE) n.s.
T.OSH (132)	2013	7.10 (±1.54)	5.40 (±2.72)	21.20 (±4.18)
T.OFS (132)	2013	7.20 (±1.13)	9.00 (±4.05)	19.30 (±3.10)
T.WOP (132)	2013	9.80 (±1.64)	8.60 (±4.03)	17.50 (±3.05)
CONV (132)	2013	9.20 (±1.72)	7.40 (±3.40)	15.60 (±4.30)
		(F = 0.81; *df* = 3; *p* = 0.49)	(F = 0.20; *df* = 3; *p* = 0.89)	(F = 0.42; *df* = 3; *p* = 0.73)
T.OSH (132)	2014	8.17 (±1.13)	7.91 (±4.01)	8.08 (±2.02)
T.OFS (132)	2014	7.67 (±1.19)	8.83 (±2.93)	5.33 (±1.15)
T.WOP (132)	2014	9.33 (±1.16)	6.58 (±1.69)	6.25 (±1.55)
CONV (132)	2014	6.50 (±1.18)	9.33 (±4.17)	5.08 (±1.55)
		(F = 1.01; *df* = 3; *p* = 0.39)	(F = 0.12; *df* = 3; *p* = 0.94)	(F = 0.72; *df* = 3; *p* = 0.54)

n.s.—not significant in the column by Tukey's test at 5% significance ($p > 0.05$). (n): Repetitions' number.

Table 4. The mean percentage of coffee leaf miner infestation (%CLM), predation (%PRED), and parasitism (%PAR) during 2013 and 2014 within conventional and three agroecological transitions systems of coffee plants *(Coffea arabica*).

System (n)	Year	% CLM (±SE) n.s.	% PRED (±SE) n.s.	% PAR (±SE)
T.OSH (132)	2013	7.10 (±1.54)	5.40 (±2.72)	21.20 (±4.18) *
	2014	8.17 (±1.13)	7.91 (±4.01)	8.08 (±2.02)
		(F = 0.32; *df* = 1; *p* = 0.57)	(F = 0.24; *df* = 1; *p* = 0.62)	(F = 8.88; *df* = 1; *p* = 0.007)
T.OFS (132)	2013	7.20 (±1.13)	9.00 (±4.05)	19.30 (±3.10) *
	2014	7.67 (±1.19)	8.83 (±2.93)	5.33 (±1.15)
		(F = 0.07; *df* = 1; *p* = 0.78)	(F = 0.001; *df* = 1; *p* = 0.97)	(F = 20.56; *df* = 1; *p* = 0.0002)
T.WOP (132)	2013	9.80 (±1.64)	8.60 (±4.03)	17.50 (±3.05) *
	2014	9.33 (±1.16)	6.58 (±1.69)	6.25 (±1.55)
		(F = 0.05; *df* = 1; *p* = 0.81)	(F = 0.24; *df* = 1; *p* = 0.62)	(F = 11.95; *df* = 1; *p* = 0.002)
CONV (132)	2013	9.20 (±1.72)	7.40 (±3.40)	15.60 (±4.30) *
	2014	6.50 (±1.18)	9.33 (±4.17)	5.08 (±1.55)
		(F = 1.75; *df* = 1; *p* = 0.19)	(F = 0.12; *df* = 1; *p* = 0.73)	(F = 6.29; *df* = 1; *p* = 0.02)

* Significant within a system between the two years by the F test at $p < 0.05$. (n): Repetitions' number.

The results showed that in all systems studied, coffee leaf miners occurred throughout the study period but did not reach the control level (30%) (Figure 3). The highest rate of infestation in the conventional system was observed in October 2013 and December 2014, reaching values of 20% and 17%, respectively. The highest predation rates (27% and 53%)

were observed in November 2013 and April 2014. The highest parasitism rates (38% and 35%) were observed in October 2013 and November 2013 (Figure 3C).

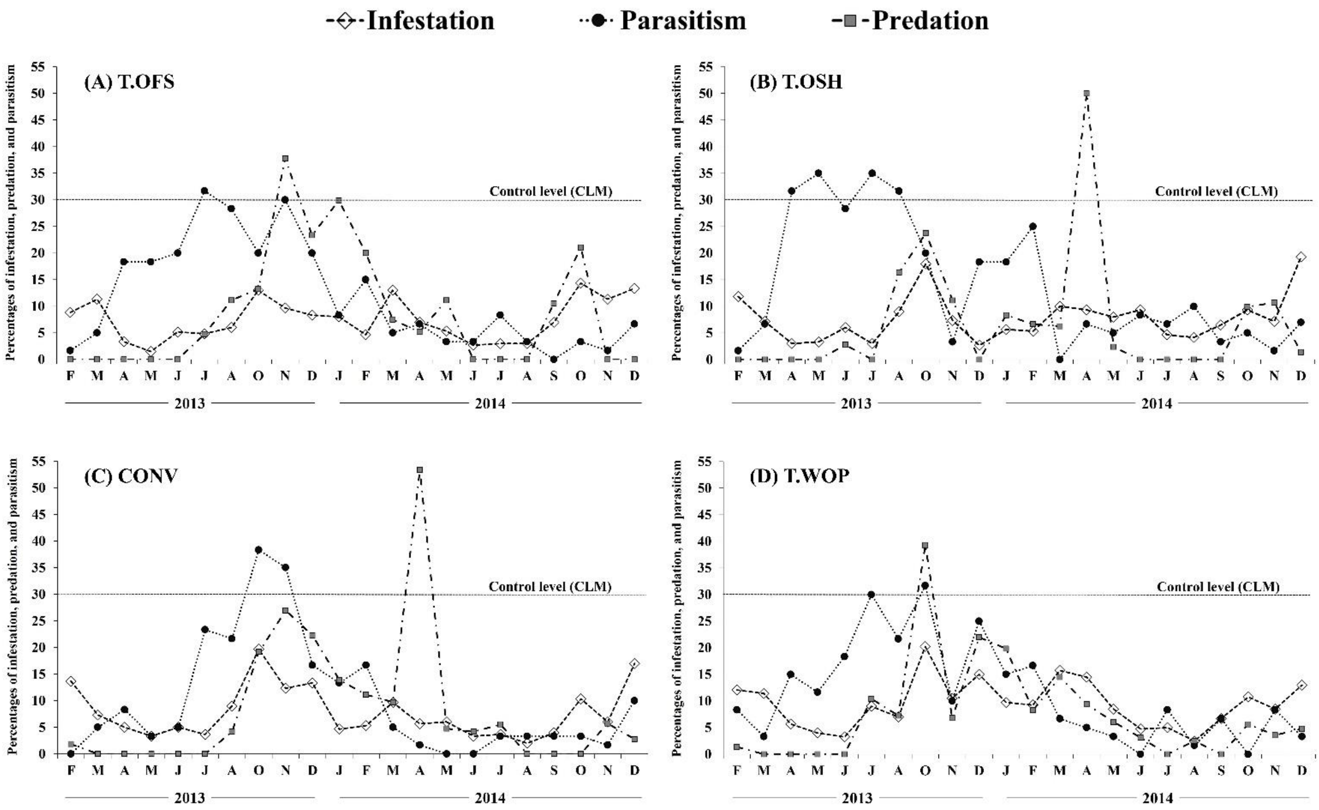

Figure 3. Variation in the percentages of infestation, predation, and parasitism of the coffee leaf miner, relative to the control level, in the conventional system (CONV) and the systems in transition to organic shaded (T.OSH), organic full-sun (T.OFS), and without pesticide (T.WOP). Campo do Meio and Guapé, MG, Brazil, February 2013 to December 2014.

In T.OSH, the highest infestation rate was observed in October 2013 and December 2014, reaching values of 18% and 19%, respectively. The highest predation rates (24% and 50%) were observed in October 2013 and April 2014. The highest parasitism rates (32% and 35%) were found in April and August 2013 and May and July 2013 (Figure 3B).

In T.OFS (Figure 3A), the highest infestation rates were observed in October 2013 and October 2014, reaching values of 13% and 14%, respectively. The highest predation rates (38% and 30%) were observed in November 2013 and January 2014. The highest parasitism rates (32% and 30%) were observed in July 2013 and November 2013.

T.WOP showed the highest infestation rate in October 2013 and March 2014, reaching values of 20% and 16%, respectively. The highest predation rates (39% and 22%) were seen in October 2013 and December 2013. The highest parasitism rates (30% and 32%) were found in July 2013 and October 2013 (Figure 3D).

4. Discussion

4.1. Diversity, Richness, and Abundance of Parasitoids

In the present study, the overall results showed that all parasitoids that emerged from intact mines of the coffee leaf miner belonged to the families Braconidae and Eulophidae. Similar results have been reported not only in Minas Gerais state [22,26,30,43] but also in other states of Brazil [25,44,45].

At the species level, the braconids *O. niger* and *S. reticulatus* were the most abundant, and among the Eulophidae, the most numerous were *P. coffeae*, *C. coffeellae*, and *Cirrospilus* sp. Previous studies carried out in Minas Gerais demonstrated that different species of parasitoids might be more or less abundant in lesions caused by the coffee leaf miner in coffee plantations. Thus, a high abundance of the eulophids *C. coffeellae*, *Proacrias* sp, *Cirrospilus* sp. 2, and *Horismenus* sp. as well as the braconids *S. letifer* and *S. reticulatus* has been reported [22,30,46]. Furthermore, the main parasitoid species were found to be generally the same but present in different proportions in other Brazilian states. In the state of São Paulo, aside from the predominance of the genera *Closterocerus* and *Proacrias*, [47] reported that among the braconids, the more abundant specimens belonged to the genera *Stiropius* and *Orgilus.*

In general, as observed in the present study in southern Minas Gerais, Eulophidae has greater species richness than Braconidae and it can be assumed that there are no families or species of parasitoids that are numerically predominant throughout the country. Most likely, the greater richness of Eulophidae is because most of its representative genera are more generalist and those of Braconidae are more specialized [38].

Considering the different production systems studied, T.OSH and T.OFS had the highest species richness (eight) of *L. coffeella* parasitoids, the majority being eulophids. The other systems, T.WOP and CONV, had one fewer Eulophidae species. In addition, the abundances of Braconidae and Eulophidae were numerically higher in T.OSH than in the other systems, and differences were also observed for *O. niger*, which was more abundant in T.OSH than the other systems but without significant differences. Greater larval parasitism by Braconidae has been reported in complex agricultural landscape systems presenting higher plant diversity compared to simple landscapes [48]. In this context, T.OSH can be considered a more complex but balanced system that offers more resources to natural enemies. Indeed, areas with shade trees provide important ecological services in the coffee agroecosystem, such as the provision of organic matter, increasing nitrogen in the soil microclimatic regulation, weed suppression, refuges for natural enemies, and many other ecological functions [15].

The absence of differences in the abundance between the two species of braconids, *O. niger* and *S. reticulatus,* mainly encountered across the analyzed cultivation systems was also reported for shaded and full-sun coffee systems [49] as well as in organic and conventional coffee plantations [43]. However, and in contrast with the latter, [45] investigated the parasitoid species of the coffee leaf miner in farms under an organic versus conventional system and concluded that these same species occurred in both systems, but with a greater abundance of *O. niger* in the conventional one. The greater abundance of *O. niger* was associated with the greater infestation of *L. coffeella* in the conventional system. This result indicates that the differentiation of coffee plantation systems based on the richness or abundance of braconids that parasitize *L. coffeella* is difficult in most cases.

Nevertheless, in the south of Minas Gerais, where this study was conducted, the braconids *S. reticulatus* and *O. niger*, due to their abundance and dominance, deserve attention when developing conservation or applied biological control programs for *L. coffeella* population control, regardless of the cultivation system used by the coffee growers [22]. A biological control conservation strategy requires adopting techniques to increase diversification in these habitats to ensure the preservation and flourishing of natural enemies as control agents.

Regarding the eulophids, the parasitoid *P. coffeae* was found to be numerically more abundant than the other species in all the systems studied here. This parasitoid was also recorded in other Brazilian states, standing out for the higher proportion of parasitism among Eulophidae in Paraná [50] and being the most abundant species in the municipality of Luiz Eduardo Magalhães in Bahia state [44]. In spite of that, the same parasitoid was previously reported to be similarly abundant in conventional, no-pesticide, and organic systems in the south of Minas Gerais [22] as well as in conventional and organic systems in the state of São Paulo [45].

The combined results from the literature and this study show that the quantitative or qualitative predominance of a species from one family over another must be associated with several factors, including physical (climate) and biological (alternative hosts and adult resources) factors, intra-guild predation, surrounding vegetation, tree canopy opening factors, and other factors that have not yet been fully elucidated.

4.2. Infestation, Predation, and Parasitism of Coffee Leaf Miner

Independently of the year, the presence of the coffee leaf miner was observed throughout the evaluation period in all the cultivation systems but without reaching the control levels as reported elsewhere for Minas Gerais [51,52], confirming that the pest is well established in the region. In general, this pest's population peaks coincide with the dry periods of the year; in the southeastern region of Brazil, the pest usually begins appearing between June and August and shows a population peak in October. The leaf miner's population fluctuation is related to their feeding habits, food availability, and abiotic factors, and any disturbance in the environment can affect the quantity and quality of food available and, consequently, the species population fluctuation [53].

During 2013, the highest infestation rate was observed in October in all systems, while in 2014, the infestation rate in the CONV and T.OSH systems was highest in December, in March in the T.WOP system, and in October in the T.OSH system. These findings confirm the variation in the infestation rate without reaching the level of damage observed by [31] who sampled the coffee leaf miner during a multi-year study in conventional coffee plantations in the south of Minas Gerais. The same was observed by [54] in coffee plantations in 2013 and 2014 in the same region. Furthermore, [22,55] evaluated the production of agroecological coffee in the south of Minas Gerais and the percentage of coffee leaf miner infestation in four cultivation systems (conventional, organic, organic-mineral, and natural agroforestry) and concluded that the infestation rate was similar between the studied systems, with slightly higher values in the natural agroforestry system. Similarly, in Nicaragua, no differences between the levels of damage of *L. coffeella* in coffee plants grown in agroforestry systems and coffee plants grown under the full sun were detected [56].

Differences in the population fluctuations of this pest between the studied systems have been reported to be mainly related to the local climatic conditions (precipitation and temperature) [53], although such a relation was not clearly confirmed here. [43] evaluated the infestation and predation of the coffee leaf miner in organic and conventional coffee plantations in the municipality of Santo Antônio do Amparo, MG, and observed a reduction in pest infestation starting at the beginning of the rainy season. According to the author, rainfall may have contributed to the intense reduction in the population of coffee leaf miner caterpillars. Other studies also cite rainfall as an important factor of the natural mortality of the coffee leaf miner as heavy rainfalls are believed to cause the death of caterpillars by drowning inside the mines [51,57].

Additionally, the action of natural enemies (predators and parasitoids) was reported to also act on the dynamics of the infestation of leaf miner singly or in combination with climatic factors [52]. Although high levels of parasitism and predation of coffee leaf miner larvae were observed in the present study after pest infestation peaks, no significant difference in parasitism could be found between the T.WOP, CONV, T.OFS, and T.OSH systems either in 2013 or in 2014. In the evaluated period, there was a marked reduction in coffee leaf miner infestation in coffee plantations in the studied areas, evidencing the contribution of these natural enemies, along with climatic factors, to the regulation of the pest population in these areas. The highest levels of predation of coffee leaf miner larvae were observed after pest infestation peaks.

In the CONV system, insecticides were used to control pests when necessary, but pesticide applications did not coincide with the phenology of the parasitoid, and systemic insecticides applied via soil were used which have less action on natural enemies [25]. These conventional production areas have surrounding vegetation with forests that can be sources of refuge and resources for natural enemies. In addition, conventional coffee trees with different surrounding vegetation and in monoculture are favorable environments for the occurrence of parasitoid hymenoptera of the coffee leaf miner.

Furthermore, the percentage of parasitism in intact mines tended to be higher in T.OSH in 2013 and 2014. This result was somewhat expected considering that this system has greater richness and abundance of *L. coffeella* parasitoids and therefore greater potential for parasitism. Organic crops and agroforestry systems are known to generally show more constant and higher mean parasitism rates and greater diversity of parasitoids than conventional or monoculture coffee crops [30,50]. In addition, a more complex cultivation system (natural agroforestry) can lead to an increase in the level of parasitism of *L. coffeella*, whereas for systems more similar in terms of plant diversification (conventional, no-pesticide, and organic), differences are not observed [22].

5. Conclusions

In the coffee plantations studied, the coffee leaf miner population is well established and does not cause economic damage; the action of predatory wasps and parasitoids—in addition to climatic factors—might contribute to the regulation of pest infestation. Conventional coffee plantations and those in transition to organic shaded, full-sun, and no-pesticide systems are favorable environments for the maintenance and preservation of predatory wasps and hymenopteran parasitoids of coffee leaf miners.

The transition from conventional to agroecological coffee cultivation in the south of Minas Gerais does not lead to an increase in coffee leaf miner infestation. The parasitoids *O. niger* and *S. reticulatus* are promising and well-adapted species for coffee-growing areas, though they require more in-depth studies for use in applied biological control programs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15010002/s1. Figure S1: A map indicating the Sampling sites and the location of the plots; Figure S2: NMDS analysis and the general result of ANOSIM; Figure S3: Script (in part) showing the GLM model selection; Figure S4: Chao Estimator; Figure S5: Cumulative monthly precipitation (mm) and average monthly temperature (°C); Table S1: ANOSIM table of analysis.

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