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Diversity of wasps (Hymenoptera: Vespidae) in conventional and organic guarana (*Paullinia cupana* var. *sorbilis*) crops in the Brazilian Amazon

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Abstract

Diversity of wasps (Hymenoptera: Vespidae) in conventional and organic guarana (*Paullinia cupana* var. *sorbilis*) crops in the Brazilian Amazon. The present study aimed to determine the diversity of wasp species associated with the guarana crop and the difference in composition of species associated to organic and conventional crops, as well as among environments established in each management (adjacent forest, crop edge and guarana crop). We collected 977 individuals and 59 species, in 23 genera of Vespidae, sixteen of Polistinae (52 species) and seven Eumeninae (seven species). *Polybia* was the most abundant and rich genus with 553 specimens and 15 species, followed by *Agelaia* (139, nine) and *Protopolybia* (103, five). In organic management crop, 686 individuals allocated in 18 genera and 47 species were collected, whereas in conventional management crop 291 individuals allocated in 18 genera and 41 species were collected. According to the three sampling points, in both management types, the edge of the crop field shows the highest abundance of wasps with a total of 519 individuals allocated in 19 genera and 45 species. Given the intense use of both environments (forest and crop) by the wasps, it is important to grow crops in regions near native forests, where the chances of social wasp colonies to be founded are increased.

Introduction

The replacement of natural areas by monocultures and pastures is resulting in widespread local and global biodiversity loss. Besides habitat fragmentation, the use of pesticides and insecticides reduces the diversity of pollinators (Durigan et al., 2007; Lindenmayer et al., 2013; Pimentel et al., 1992). Pimentel et al. (1992) assert that it is important to conserve biological diversity in agricultural ecosystems, which, along with human settlements, cover approximately 95% of terrestrial environment.

Farmland conservation programs aim at improving the value of agricultural landscapes for biodiversity, and a key conservation strategy of these programs is the retention of remnant natural vegetation (Kleijn et al., 2011; Phalan et al., 2011). The maintenance of the edge vegetation is considered one of the most effective measures for sustaining insects diversity in crops (Attwood et al., 2008; Tscharntke et al., 2008). It allows native insects to persist in the transformed landscape by providing undisturbed refuge and supplementary resources within the agricultural mosaic (Benton et al., 2003; Duelli & Obrist, 2003; Gaigher et al., 2015).

In the Amazon region, guarana (*Paullinia cupana* var. *sorbilis* (Mart.) Ducke) is one of the most cultivated plants; it is a native Amazonian plant belonging to the Sapindaceae family. According to IBGE (2013), the planted area in Brazil is 14.952 hectares and the Amazonas state is one of the largest producers. Currently, there is a great demand for organic products of guarana (Tavares et al., 2003; Tavares & Garcia, 2009). However, the absence of basic studies (biology and ecology) and data about the composition of fauna biodiversity, especially of natural enemies as as predatory and parasitoids Hymenoptera, make it impossible or delay the development of alternative techniques to biodiversity conservation and pest control.

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The Vespidae family occurs over a wide range of habits and presents varying levels of social complexity, serving as regulators of other insect populations (Prezoto, 1999; Carpenter & Marques, 2001), as well as pollinators (Sühs et al., 2009). The most part of protein acquired by wasps in their foraging comes from the capture of caterpillars, the main group of insects that feed on cultivated plants (Prezoto et al., 2008). Thus, wasps play a major role in agricultural systems (Carvalho & Souza, 2002).

The most common subfamilies in the Brazilian Amazon are Polistinae and Eumeninae. The social Polistinae comprises 26 genera and 958 described species widely distributed in the Neotropical region (Pickett & Carpenter, 2010). The Polistinae social wasps are important components of Neotropical ecosystems due to their ubiquity and diversity, as well as their complex interactions with other organisms (Silveira, 2002). The species composition of a determined area is an important factor on which to base comprehensive scientific studies of its ecological characteristics (Humprhey et al., 1999). The highest diversity of Polistinae is found in Brazil (319 species recorded) and Silveira (2002) pointed out that 200 species were recorded in the Brazilian Amazon. The Eumeninae solitary wasps include 3.579 species, the subfamily with the highest number of species among vespid wasps and about 300 species have been recorded from Brazil (Carpenter & Garcete-Barrett, 2002; Pickett & Carpenter, 2010).

In this context, the present study aimed to determine the diversity of wasp species associated with the guarana crop and the difference in composition of associated species to organic and conventional crop, as well as among different environments in each management (adjacent forest, crop edge and guarana crop).

Material and Methods

Study area

The study was conducted within the experimental fields of Western Amazon EMBRAPA (Brazilian Enterprise of Agriculture and Cattle-Raising), in Manaus, Amazonas, Brazil, where there are two guarana crop fields, one under organic management (2°53'29.14"S / 59°58'45.80"W) and the other under conventional management system (2°53'42.18"S / 59°59'10.58"W).

The conventional management crop was established in 1986 in an area of 1.6 ha with 710 plants cultivated in a spacing of 5 m x 5 m. In this crop field, the application of insecticide happened only once, on January 1st, 2013. The organic management crop was established in 2003 in an area of 3.9 ha with 1,595 plants cultivated in a spacing of 5 m x 5 m.

Sampling design

In this study, we used two distinct and usual sampling methods, the *Malaise* traps and the *Möericke* traps. With Malaise traps (Townes model), the wasps were collected in a container located in the upper region of the trap, which had alcohol 70%. The *Möericke* trap consists of a yellow container measuring 25 cm of length x 15 cm width x 5 cm of height, and water solution with 2 ml of neutral detergent.

In each sampling occasion, which happened at biweekly intervals, the traps were set up for four days, and the water from the *Möericke* trap was replaced every 24 hours in order to avoid loss and/or deterioration of biological material. A total of 12 samplings were conducted from September 2012 to February 2013.

In each crop field (conventional and organic management), a diagonal sampling line was established along the three sampling points: 1) in the interior of the crop field; 2) at the edge of the crop field, and 3) outside the crop field, in the adjacent forest area. The distance between each sampling point was 60 m. At each point, one *Malaise* trap was installed. The distance between conventional and organic management crops is approximately 5 km.

Data analysis

The specimens of Vespidae were sorted and identified at the Hymenoptera Laboratory of the National Institute of Amazonian Research (INPA). The vouchers were deposited at INPA's Invertebrate collection.

In this study, several measurements (samples) were made in the same experimental units (organic and conventional crop fields) over a period of time. Such data are called 'repeated measures' (Crowder & Hand, 1990; Davis, 2002; Gotelli & Ellison, 2004).

In order to verify if the variables 'type of crop' (conventional and organic management), 'sampling techniques' (*Malaise* and *Möerike*), and 'sampling points' (adjacent forest, edge and interior of crop field) influenced wasps species richness, we performed an analysis of variance (ANOVA) for repeated measures, since the samples were taken in the same crop fields across time. Similarly, we performed the ANOVA with repeated measures to test the influence of the variables 'type of crop field', 'sampling techniques', and 'sampling points' on the species composition of wasps. With both metrics (richness and composition) we tested whether there was an interaction between samples (over time) and other variables used in ANOVA models. If required, ANOVA analysis was followed by the post hoc Tukey multiple-range test (Yandell, 1997). The dimensionality of wasps abundance data was reduced using Nonmetric Multidimensional Scaling (NMDS, Minchin, 1987) based on the Bray-Curtis dissimilarity index.

Additionally, we calculate the Shannon diversity index (H') and the Pielou equitability index (J'). All analyses were conducted in the free software R, version 3.1.0 (R Development Core Team, 2014), using the Vegan package (Oksanen et al., 2013).

To calculate the frequency of the species, which is the proportion of individuals of a species in relation to all individuals in the sample, we used this formula: $F = n/N \times 100$, where F = frequency (in percentage); n = number of individuals of each species and; N = total number of individuals.

Table 1. Vespidae species collected in guarana organic and conventional management and sampling points, in the Brazilian Amazon. Forest = adjacent forest, Edge = edge of the crop and Interior = interior of the crop. FR (%) = Frequence.

Taxon	Conventional guarana crop			Organic guarana crop			TOTAL	FR (%)
	forest	edge	interior	forest	edge	interior		
Agelaia angulata Fabricius, 1804	9	0	5	18	0	0	32	3.3
Agelaia cajennensis (Fabricius, 1798)	0	0	0	0	1	1	2	0.2
Agelaia centralis Cameron, 1907	0	2	0	0	0	0	2	0.2
Agelaia constructor de Saussure, 1854	3	0	1	2	0	1	7	0.7
Agelaia flavipennis (Ducke, 1905)	0	0	2	2	1	0	5	0.5
Agelaia fulvofasciata DeGeer, 1773	2	2	3	2	10	5	24	2.5
Agelaia myrmecophila Ducke, 1905	1	1	1	1	1	1	6	0.6
Agelaia pallipes Olivier, 1792	0	1	1	8	12	38	60	6.2
Agelaia testacea Fabricius, 1804	0	0	0	1	0	0	1	0.1
Angiopolybia obidensis Ducke, 1904	0	0	0	4	0	0	4	0.4
Angiopolybia pallens Lepeletier, 1836	2	1	0	14	5	2	24	2.5
Angiopolybia paraensis Spinola, 1851	0	7	2	3	4	0	16	1.6
Apoica pallida Olivier, 1792	0	0	0	0	0	1	1	0.1
Apoica strigata Richards, 1978	0	0	0	1	0	0	1	0.1
Brachygastra billineolata Spinola, 1841	0	2	1	0	0	0	3	0.3
Chartergellus amazonicus Richards, 1978	0	0	1	0	2	0	3	0.3
Chartergellus jeannei Andena & Soleman, 2015	0	2	0	0	1	0	3	0.3
Chartergus chartarius Olivier, 1791	0	0	0	0	1	0	1	0.1
Charterginus xanthura (de Saussure, 1854)	0	0	0	0	3	0	3	0.3
Hypalastoroides sp.1	1	0	0	0	0	0	1	0.1
Leipomeles dorsata Fabricius, 1804	0	1	0	0	0	0	1	0.1
Leipomeles spilogastra Cameron, 1912	0	0	1	0	0	0	1	0.1
Metapolybia cingulata Fabricius, 1804	0	1	0	0	0	1	2	0.2
Mischocyttarus labiatus Fabricius, 1804	3	3	1	0	1	1	9	0.9
Mischocyttarus rotundicollis Cameron, 1912	0	1	0	0	0	1	2	0.2
Mischocyttarus sp.1	0	0	0	0	0	3	3	0.3
Montezumia sp.1	0	0	0	0	0	1	1	0.1
Omicron sp.1	0	1	0	0	1	0	2	0.2
Pachodynerus sp.1	0	4	0	1	2	1	8	0.8
Pachymenes sp.1	3	6	3	2	10	5	29	3.0
Parachartergus smithii de Saussure, 1854	0	0	0	0	4	1	5	0.5
Parachartergus griseus (Fox, 1898)	0	0	1	0	0	0	1	0.1
Parachartergus sp.1	0	0	1	0	0	0	1	0.1
Polistes geminatus Fox, 1898	0	1	1	0	0	0	2	0.2
Polistes pacificus Fabricius, 1804	1	10	16	2	0	0	29	3.0
Polybia belemensis Richards, 1970	0	2	1	0	14	5	22	2.3
Polybia bistriata Fabricius, 1804	0	0	0	0	3	5	8	0.8
Polybia dimidiata Olivier, 1791	0	1	0	0	0	0	1	0.1
Polybia dubitata Ducke, 1910	0	0	1	1	0	0	2	0.2
Polybia emaciata Lucas, 1879	1	1	1	0	4	2	9	0.9
Polybia gorytoides Fox, 1898	0	0	0	0	1	0	1	0.1
Polybia ignobilis Haliday, 1836	0	1	1	0	0	0	2	0.2
Polybia jurinei de Saussure, 1854	0	0	0	0	6	10	16	1.6
Polybia liliacea Fabricius, 1804	3	2	1	17	23	4	50	5.1
Polybia occidentalis Olivier, 1791	3	24	18	17	107	13	182	18.7

Table 1. Vespidae species collected in guarana organic and conventional management and sampling points, in the Brazilian Amazon. Forest = adjacent forest, Edge = edge of the crop and Interior = interior of the crop. FR (%) = Frequence. (Continuation)

Taxon	Conventional guarana crop			Organic guarana crop			TOTAL	FR (%)
	forest	edge	interior	forest	edge	interior		
Polybia parvulina Richards, 1970	0	0	0	0	5	0	5	0.5
Polybia rejecta (Fabricius, 1798)	1	35	53	10	86	62	247	25.3
Polybia sericea Olivier, 1792	0	0	1	0	0	0	1	0.1
Polybia velutina Ducke, 1907	0	1	0	0	0	0	1	0.1
Polybia (*Myrapetra*) sp.1	0	3	0	1	1	1	6	0.6
Protopolybia bituberculata Silveira & Carpenter, 1995	0	5	2	2	21	4	34	3.5
Protopolybia chartergoides Gribodo, 1891	0	2	0	0	2	3	7	0.7
Protopolybia minutissima (Spinosa, 1851)	0	7	1	1	35	12	56	5.8
Protopolybia picteti (Cameron, 1907)	0	2	0	0	2	1	5	0.5
Protopolybia sp.1	0	0	0	0	1	0	1	0.1
Pseudodynerus sp.1	0	1	0	0	3	0	4	0.4
Pseudopolybia vespiceps de Saussure, 1863	0	0	0	0	1	1	2	0.2
Synoeca virginea Fabricius, 1804	0	2	1	2	6	2	13	1.3
Zethus sp.1	0	0	1	0	4	2	7	0.7
Total of individuals	33 (3.4)	135 (13.8)	123 (12.6)	112 (11.4)	384 (39.3)	191 (19.5)	977	100

Results

We collected 977 wasps specimens. The subfamily Polistinae was the most abundant with 925 specimens, while Eumeninae was represented by 52 individuals. *Polybia* Lepeletier was the most abundant genus, followed by *Agelaia* Lepeletier and *Protopolybia* Ducke with 553, 139 and 103 specimens, respectively. We determined 59 species of Vespidae in 23 genera, 52 species in sixteen genera of Polistinae and seven species in seven genera of Eumeninae. Again, *Polybia*, *Agelaia* and *Protopolybia* were the most rich, with fifteen, nine, and five species, respectively (Table 1).

Polybia rejecta (Fabricius, 1798) was the most abundant and frequent (n = 247; 25.3%) species in guarana crop, followed by *Polybia occidentalis* Olivier, 1791 (n = 182; 18.7%). Our collections include species that merit particular notice; we collected species that represent significant records because they have not been recorded by recent surveys in Amazonas state: *Chartergellus amazonicus* Richards, 1978, *Chartergus chartarius* Olivier, 1791, *Charterginus xanthura* (de Saussure, 1854), *Leipomeles dorsata* Fabricius, 1804, *Leipomeles spilogastra* Cameron, 1912, *Protopolybia minutissima* (Spinosa, 1851), *Protopolybia picteti* (Cameron, 1907).

In organic management crop, we collected 686 individuals allocated in 18 genera and 47 species. In conventional management crop, we collected 291 individuals allocated in 18 genera and 41 species. *Polybia* was the most abundant and species-rich genus, in both crops. The Shannon diversity index was similar for both managements after the species richness was approximated (H '= 2.71). Regarding the Pielou equitability index, an almost homogeneous distribution between the species both in the conventional management (J '= 0.72) and in organic (J' = 0.70) was observed, except for *P. occidentalis* and *P. rejecta*, whose number of individuals were higher than the other species.

In the three sampling points, we collected 519 individuals allocated in 19 genera and 45 species in the edge of the crop, 313 individuals allocated in 18 genera and 43 species in the interior of the crop and 145 individuals allocated in 10 genera and 25 species in the adjacent forest. In both management types, the edge of the crop field showed the highest number of individuals.

Differences in richness of Vespidae were detected between the organic and conventional crop ($ANOVA_{(1,84)}$ F=29.078; p<0.001*), and among sampling points - adjacent forest, edge and interior of crop field ($ANOVA_{(1,84)}$ F=27.931; p<0.001*). The other variables did not affect the richness of wasps. The variable sample (over time) showed no interaction effect with the others variables used in the model, that is, it did not affect the detection of the individual effect of tested variables (Fig 1; Table 2).

Table 2. Analysis of variance with repeated measure results for a model using type of crop field, sampling point, sampling techniques and samples for Vespidae richness. Significance level: p< 0.001*.

Variables	Degrees of freedom	F value	p value
Type of crop field	1	29.078	<.0001*
Sampling point	2	27.931	<.0001*
Samples	11	0.781	0.655
Interaction between type of crop field and samples	11	0.795	0.643
Interaction between sampling point and samples	22	0.780	0.714

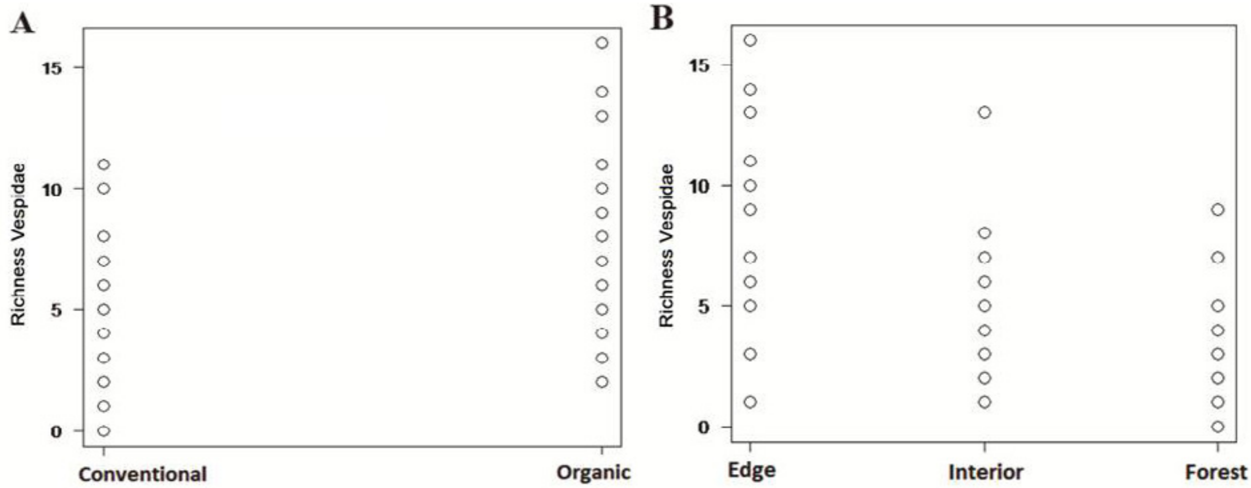

Fig 1. The Vespidae richness in guarana organic and conventional management (A) and sampling points (B), in the Brazilian Amazon.

On the other hand, differences in composition of Vespidae species were not detected between the organic and conventional crop ($ANOVA_{(1,84)}$ F=0.972; p<0.3286), or among sampling points ($ANOVA_{(1,84)}$ F=1.065; p<0.352). Therefore, the variable sample (over time) showed no interaction effect with the others variables used in the model, that is, it did not affect the detection of the individual effect of tested variables (Tab. 3).

Table 3. Analysis of variance with repeated measure results for a model using type of crop field, sampling point, sampling techniques and samples for Vespidae composition. Significance level: p< 0.001*.

Variables	Degrees of freedom	F value	p value
Type of crop field	1	0.972	0.329
Sampling point	2	1.065	0.352
Samples	11	0.782	0.655
Interaction between type of crop field and samples	11	0.795	0.643
Interaction between sampling point and samples	22	0.780	0.714

Discussion

Wasp surveys are lacking in agricultural ecosystems in Brazil, especially in the Amazon Region, making this a pioneer study for the guarana crop. The Vespidae richness and composition obtained in this study were higher than previous studies conducted in different crops. For example, in an area of Silvipastoral culture of Embrapa Dairy Cattle Research Center in Minas Gerais, a total of 205 social wasp specimens, distributed in 13 morphospecies and four genera were captured (Auad et al., 2010). In forest fragments with different surrounding matrices of sugarcane and *Citrus* crops in São Paulo, a total of 1460 social wasp specimens, distributed in 29 morphospecies and 10 genera were captured (Tanaka Junior & Noll, 2011). No studies including the Eumeninae were conducted so far.

However, comparing this study with protected areas or reserves in Amazon rainforest, we noticed a lower species richness in guarana crop. For example, in an inventory at Ducke Reserve in Amazonas state, 58 species of social wasps (Polistinae) in 13 genera were collected (Somavilla et al., 2014) and in Caxiunã Reserve, in Para state, 65 species in 12 genera of Polistinae were collected (Silva & Silveira, 2009), both with a higher species richness than this inventory in cultivated area, but with a lower number of genera.

Polybia rejecta and *Polybia occidentalis* are two very abundant species in Amazon region, most often recorded in open areas but rarely captured in close rainforest (Somavilla et al., 2014). Furthermore, these two species found colonies in open areas, such as the edge of guarana crop, enhancing the collection of these wasps in crops.

The high abundance of *Agelaia* and *Polybia* registered in the current and other studies indicates that species of these genera find it easy to colonize several different types of microhabitats due to their protected nests, method of foundation and great number of individuals, which gives their colonies greater chances of success (Hermes & Köhler, 2004). In this study, their abundance was higher in adjacent forest and interior of the crop, showing a great capacity for dispersion, unlike the results of others studies where usually the uniform environments and interior of the crops lower their abundance, and suggests that these species encounter barriers to use resources outside the better-conserved environments (Klein et al., 2015).

The adjacent forest and the edge of cultures is very important for maintaining the biological diversity in agricultural ecosystems. The Vespidae richness and composition in a crop depend on the diversity of vegetation within or in the edge, presence of only one or various cultures, the intensity of management, the distance of the natural vegetation and the presence of food (Gaigher et al., 2015). The wasps use directly vegetation to building their nests and search for food, for this, vegetation improves the chances of social wasp colonies to be successfully founded because of their ability to migrate between these habitats.

The sampling points within the forest and within the interior of the crop, for both conventional and organic crops, were similar in terms of composition of Vespidae. This similarity may have reflected the environment conditions, which present preserved areas in the surroundings, composed of several different plants from the main crop. These areas provide shelter and food for the wasps (Altieri & Nicholls, 2004) and increase the concentration and distribution of wasps inside the crop.

The abundance of Vespidae was higher in organic management (70%) when compared to conventional. The greatest abundance can may be linked to the fact that no influence of chemical pesticides and fertilizers, which allows the entry of wasps inside of the crop. The wasps, in turn, enter the crop in order to establish a colony or to get resources for their survival, such as food or material needed to build their nests, which explains the presence of a lot of wasps social this type of management.

In the conventional crop field, the edge and the interior crop had similar richness. Surprisingly, the most similar environments in the organic crop field were the adjacent forest and the edge. These results have differed from the expected (adjacent forest and edge of crop field more similar to each other in the conventional crop system; and edge and interior of crop field in the organic crop system). Perhaps, these results indicate that it is occurring the entry of wasp species into in interior crop for search food and nesting resources.

Concerning the two sampling methods, the use of several sampling methods increases the chances of sampling all potential niches (Longino et al., 2002). However, it also increases the amount of time and money employed during the studies. Thus, for the purpose of surveying Vespidae fauna in guarana crops, it is ideally recommended the use of *Malaise* trap, for the *Möericke* traps do not have an efficiency for the collection of Vespidae.

This study highlights the importance of conserving natural fragments for maintaining biodiversity in agricultural landscapes, which supports existing set-aside programs. This is an important consideration in a region where high-value crops and biodiversity-rich natural land coincide (Fairbanks et al., 2004) and where the potential for natural habitat conversion for agricultural expansion is high (Underwood et al., 2009; Viers et al., 2013; Gaigher et al., 2015).

Therefore, it is important to grow crops in regions near native forest, given the activity of the wasps in both environments. Native forest areas characterized by higher plant diversity indices have a greater abundance of food resources and places for shelter for social and solitary wasps (Townsend et al., 2006), improving the chances for social wasp colonies to be founded.

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