PROJECT TITLE:

Project 4.07. Diversity of Hymenoptera parasitoids, predators and pollinators in Cerrado e Pantanal and Seletivity of Insecticides prototypes from plants on these Hymenoptera.

HYPOTHESIS

1. There is a great abundance of different species of plants from Cerrado Biome and the Pantanal with insecticidal properties;
2. There is a great diversity and richness of Hymenoptera parasitoid, predator and pollinator surrounding Cerrado area, in Campo Grande, MS. These are more plentiful in fragment of Cerrado than in pasture area;
3. Malaise and Moericke traps, used to collect insects in methodology adapted for agricultural experiments and methodology of control of dengue by Zoonosis Control Center (CCZ) are suitable for assessment of selectivity of insecticides in the field;
4. The prototype insecticide based on the liquid of cashew nuts (LCC), composed mostly of anacardic acid 150 ml / ha (w/v) and based on Sodium ricinoleate, developed to control *Aedes aegypti*, this doesn't have an effect on the population of hymenoptera parasitoids, predators and pollinators, in field conditions;
5. There is a great diversity and richness of Hymenoptera parasitoid, predator and pollinator in the Pantanal. There are various environments such as Paratudal, Cangiqueral, Espinheiral and Riparian which have different diversity and richness ;
6. The diversity and richness of Hymenoptera parasitoids, predators and pollinators in different kinds of environment during dry and wet seasons.

OBJECTIVE

To identify active ingredients in plants from Cerrado and Pantanal for use in Agriculture and Public Health;

To understand the Hymenoptera superfamilies are more abundant in Cerrado and Pantanal, which are liable to be bioindicators;

To Develop the methodology, to evaluate the deleterious effect, or selectivity, of insecticides on non target insects, in field conditions;

To evaluate the selectivity of prototype insecticide from plants on Hymenoptera parasitoid, predator and pollinator, in field conditions

MATERIALS AND METHODS

First phase Hymenopteran parasitoids, predator and pollinator collected in Malaise trap, in Cerrado fragment, Campo Grande, MS.

Thirty samples were obtained, collected in six Malaise traps at the Fazenda São José, an experimental area in Campo Grande, MS, Brazil, from October to November 2010. The experimental area covers 2000m², with 11 hectares of Cerrado fragment (anthropized Cerradão) and 14 hectares of land devoid of native vegetation due to the invasion of *Brachiaria decumbens* (signal grass). Six Malaise traps were set up, three

outside the Cerrado fragment and three inside the fragment, 100 meters apart from each other. The traps inside the fragment were placed 50 meters away from the border.

Abundance and relative abundance were evaluated in the three areas of the Cerrado and pastureland over a 5-week period, with one sampling collection per week from October to November 2010. The results were compared using richness, diversity, dominance, evenness, and constancy indices, while similarity between the environments was evaluated by calculating the coefficient of similarity. Richness was evaluated by the Margalef index, diversity by the Shannon-Wiener index, dominance by the Simpson index, evenness by the Pielou index, and constancy was determined based on the Bodenheimer formula. Lastly, faunal similarity was calculated based on the Sorensen index.

The material was identified using Hanson and Gauld's (2006) identification keys for Neotropical Hymenoptera. The material under study is deposited in the Parasitoid Hymenoptera collection of the Department of Ecology and Evolutionary Biology of the Federal University of São Carlos (DCBU), São Carlos, SP, as well as at the Biological Institute (IB) in Campinas, SP, and at the Museum of Zoology of the University of São Paulo (MZUSP) in São Paulo, SP, Brazil.

Second phase (samples collected after spraying insecticides): Effect on prototype insecticide on Superfamiles and the Families Ichneumonidae and Vespidoidea (Hymenoptera). It was proven Ichneumonidae and Vespoidea were the best bioindicators based on results from past experiments.

In November, 19, 2012, in each sub experimental area two more traps were installed. After this, each sub experimental area contained three traps distributed in triangular shapes each measuring 60 meters. These triangles were situated 100 meter from the border of fragment.

The treatments, one from of each sub experimental area were: (1) control (without spraying), (2) prototype insecticide based Sodium Ricinoleate (37% ricinoleic acid), 1% p.c./ha and (3) Malathion 500 CE insecticide, 250 ml/ha. The insecticide and the prototype were sprayed around Malaise trap in 20 x 20 meter (400 m^2). They were diluted in a solution at a ratio of 200L/ha.

The spraying was done by Motorized Sprayer Backpack (which is similar to the controlling of mosquitos used by the Zoonozis Center Control, CCZ). The insecticide and prototype were sprayed at sunset, without wind and low light. The concentrations were determined by recommendation regarding the control of mosquitos and past experiments (Porto et al., 2008).

Before spraying two more samples were collected, once a week. Triage received technical support from a group of Researchers in Hymenoptera at University of São Carlos, SP, UFSCAR (Penteado-Dias, 2008).

Observations:

B.1. Effect of insecticide and prototype on Vespoidea and Ichneumonidae

B.2. Effect of insecticide and prototype on Meliponini Tribe

Data analisys

The data is being analyzed in the first phase with Dominance and Richness of species per superfamily and Family. Regarding the second phase the data is being analyzed with the ANOVA method followed by multiple comparisons of means using the Duncan test. The results will express a mean ± mean standard deviation.

Meliponini (Apidae) as in Campo Grande, MS

Malaise traps were installed in a pasture near a fragment of Cerrado, from October to December 2010. The apiforms specimens from each sample were separated in morphospecies and sent to identification.

Richness and abundance of Superfamily Hymenoptera parasitoids and predators in different phisionomy, in drought and flood, **in the Pantanal, Corumbá, MS.**

Four areas, riparian, paratudal, espinheiral and canjiqueiral were compared, in drought and flood. The samples were obtained from Malaise traps and trapped insects were collected separately and stored in glass collectors, preserved in 96% alcohol and sorted manually and identified with the aid of magnifying binocular (stereo) at the superfamily level.

RESULTS AND DISCUSSION

The Hymenoptera are one of the largest and most efficient orders of insects for biological control, but it is believed that less than 10% of their species are known. Given their abundance, they have significant effects on terrestrial environments, and are vulnerable to environmental changes. The characterization of an area in terms of species distribution, abundance and richness underpins research on natural biological control, conservation, and the impact of human actions on the environment. This work focused on discovering and listing Hymenoptera fauna in a degraded area and along the border of a fragment of Cerrado in Campo Grande, MS, Brazil, aiming to provide insights for future studies about the impact of pesticides on nontarget organisms. The most abundant Hymenoptera in these two environments were Vespoidea, except for Formicidae, followed by Chrysidoidea and Apoidea Apiformes, mostly in the Cerrado, closely followed by Ichneumonoidea, which are present in both environments. Vespoidea, Apiformes Apoidea and Ichneumonoidea were the most common in all samplings, followed by Chalcidoidea, Chrysidoidea and Apoidea Spheciformes. The superfamily Ichneumonoidea were the most richest and abundant, and together with the other aforementioned groups, can be considered good indicators in environmental impact studies.

Table 1 – Frequency of occurrence of Hymenoptera superfamilies (except Formicidae) captured in Malaise traps inside a fragment of Cerrado and its surroundings in Campo Grande, MS, Brazil, Oct-Nov 2010

Superfamily	Number of captured insects		Number of samples in which they were present	
	Frequency	%	Frequency	%
Apoidea Apiformes	618	17.9%	30	100.0%
Apoidea Spheciformes	212	6.2%	25	83.3%
Ceraphronoidea	4	0.1%	4	13.3%
Chalcidoidea	346	10.0%	29	96.7%
Chrysidoidea	798	23.2%	28	93.3%
Cynipoidea	8	0.2%	6	20.0%
Evanioidea	48	1.4%	8	26.7%
Ichneumonoidea	417	12.1%	30	100.0%

Platygastroidea	41	1.2%	18	60.0%
Proctotrupoidea	24	0.7%	12	40.0%
Vespoidea	929	27.0%	30	100.0%
Total captured insects	3445			

Table 2 – Frequency of specimens of Hymenoptera superfamilies (except Formicidae) captured in Malaise traps in two physiognomies – pastureland and Cerrado – in Campo Grande, MS, Brazil, Oct-Nov 2010

Superfamily	Pastureland		Cerrado	
	Frequency	%	Frequency	%
Apoidea Apiformes	224	15.5%	394	19.7%
Apoidea Spheciformes	124	8.6%	88	4.4%
Ceraphronoidea	1	0.1%	3	0.1%
Chalcidoidea	171	11.9%	175	8.7%
Chrysidoidea	188	13.0%	610	30.4%
Cynipoidea	4	0.3%	4	0.2%
Evanioidea	7	0.5%	41	2.0%
Ichneumonoidea	209	14.5%	208	10.4%
Platygastroidea	19	1.3%	22	1.1%
Proctotrupoidea	17	1.2%	7	0.3%
Vespoidea	477	33.1%	452	22.6%
Total captured insects	1441		2004	
Number of captured superfamilies	11		11	

Table 3 – Indices of richness, diversity, evenness and dominance of insects of Hymenoptera superfamilies (except Formicidae) captured in Malaise traps in a fragment of Cerrado and its border in Campo Grande, MS, Brazil, Oct-Nov 2010

Index	**Pastureland**	**Cerrado**
Margalef richness	3.17	3.03
Shannon-Wiener diversity	0.79	0.77
Pielou evenness	0.76	0.74
Dominance	0.19	0.20

Table 4 – Coefficient of similarity between the Hymenoptera superfamilies (except Formicidae) captured in Malaise traps in three areas in each of the two physiognomies, pastureland and Cerrado fragment, in Campo Grande, MS, Brazil, Oct-Nov 2010

	Coefficient of similarity				
	A2 Pastureland	A3 Pastureland	A1 Cerrado	A2 Cerrado	A3 Cerrado
A1 Pastureland	**0.87**	0.78	0.75	0.82	0.85
A2 Pastureland		0.84	0.71	0.75	0.81
A1 Cerrado				**0.88**	0.80
A2 Cerrado					**0.89**

Table 5 – Relative abundance (Frequency) of specimens of Hymenoptera superfamilies (except Formicidae) captured in Malaise traps in the Cerrado fragment and surroundings; Campo Grande, MS, Brazil, Oct-Nov 2010

Superfamilies	Relative abundance					
	A2 pasture-land	A2 pasture-land	A3 pasture-land	A1 Cerrado	A2 Cerrado	A3 Cerrado
Apoidea Apiformes	26.1%	6.9%	33.3%	39.4%	8.7%	23.1%
Apoidea Spheciformes	7.2%	8.6%	7.5%	4.0%	6.6%	2.9%
Ceraphronoidea	0.0%	0.0%	0.1%	0.3%	0.1%	0.1%
Chalcidoidea	9.1%	16.0%	8.5%	6.1%	9.0%	9.1%
Chrysidoidea	17.6%	17.5%	4.6%	28.1%	35.5%	26.8%
Cynipoidea	0.0%	0.5%	0.3%	0.3%	0.3%	0.1%
Evanioidea	1.5%	0.0%	0.0%	0.0%	3.1%	2.0%
Ichneumonoidea	14.8%	13.6%	11.5%	9.5%	11.4%	9.7%
Platygastroidea	0.9%	1.4%	1.2%	0.3%	2.2%	0.6%
Proctotrupoidea	0.4%	1.4%	1.2%	0.9%	0.6%	0.0%
Vespoidea	22.4%	34.0%	31.7%	11.0%	22.6%	25.6%
Total insects	369	518	726	318	871	845

Table 6 – Margalef richness index of morphospecies of Hymenoptera superfamilies (except Formicidae) captured in Malaise traps in the Cerrado fragment and along its borders; Campo Grande, MS, Brazil, Oct-Nov 2010

Superfamily	Area 1		Area 2		Area 3	
	Pasturelan d	Cerrad o	Pasturelan d	Cerrad o	Pasturelan d	Cerrad o
Chrysidoidea	4.53	3.37	3.58	5.24	1.47	5.66
Ichneumonoide a	8.31	4.22	3.94	6.64	6.63	5.66
Chalcidoidea	3.40	1.26	5.02	6.29	4.79	9.65
Proctotrupoidea	0.38	0.42	0.71	0.35	1.10	-0.33
Platygastroidea	0.38	0	0.71	2.10	1.10	0.33
Cynipoidea	-0.38	0	0	0.35	0.37	0
Ceraphronoidea	-0.38	0	-0.36	0	0	-0.33
Evanioidea	0.38	-0.42	-0.36	0	-0.37	0.33
Vespoidea	3.02	3.37	4.30	5.60	7.74	6.99
Apoidea Spheciformes	3.40	1.26	3.58	3.50	4.42	2.33
Apoidea Apiformes	3.02	1.26	2.51	2.10	2.21	2.33

Selectivity of insecticides on Ichneumonoidea and Vespoidea

There are insects, of importance to the environment, to be found within the Order Hymenoptera. Such as parasitoids and predators and pollinators, but isn't a lot of information regarding same. The intense use of synthetic insecticides can cause contamination in the environment and lead to dead non-target organism. There is a lack of information about the effect of insecticides on these Hymenoptera. Through this study we sought to characterize the Hymenoptera parasitoids, predators and pollinators and the effect of insecticide from Sodium ricinoleate compared to Malathion on non-target insects. Collections were made with Malaise traps installed in pasture area around Cerrado fragment, which remained in the field for five weeks, from November to December 2012. The products were sprayed around Malaise traps, in three repetitions each treatment. It was observed higher populations of Ichneumonoidea, Vespoidea and Chrysidoidea with greater frequency indicating that these are biomarkers, which are highly ratedregarding effect on insecticides in the environment. It was concluded that the insecticide from Sodium ricinoleate and Malathion are not selective to the Ichneumonidae one week after application. But it two weeks after treatment was not observed there were no differences between treatments and the control.

Table 7 Population of Hymenoptera (superfamily, family) collected on the border of Cerrado fragment, during one week, in Malaise trap, one week after spraying products, Campo Grande, MS.

Superfamily-Family	Control	Sodium Ricinoleate	Malathion	CV %
Braconidae	5,66 a*	5,66 a	2,33 a	13,20
Ichneumonidae	12,33 a	4,66 b	4,33 b	24,07
Vespoidea	41,33 a	37,33 a	32,33 a	37,98

*Means followed by the same letter in the same row, do not differ by Duncan test (P>0.05).

Table 8 Population of Hymenoptera (superfamily, family) collected on the border of Cerrado fragment, during one week, in Malaise trap, two weeks after spraying products, Campo Grande, MS.

Superfamily-Family	Control	Sodium Ricinoleate	Malathion	CV %
Braconidae	2,66 a	2,00 a	1,33 a	63,68
Ichneumonidae	8,66 a	2,66 a	2,66 a	30,55
Vespoidea	27,66 a	18,00 a	41,33 a	22,91

*Means followed by the same letter in the same row, do not differ by Duncan test (P>0.05).

Table 8 Population of Hymenoptera (superfamily, family) collected on the border of Cerrado fragment, during one week, in Malaise trap, three weeks after spraying products, Campo Grande, MS.

Superfamily-Family	Control	Sodium Ricinoleate	Malathion	CV %
Braconidae	1,00 a	0,66 a	1,33 a	80,29
Ichneumonidae	4,00 a	2,33 a	1,33 a	59,16

Vespoidea	14,33 a	12,66 a	18,66 a	29,07

*Means followed by the same letter in the same row, do not differ by Duncan test (P>0.05).

Meliponini (Apidae) as bioindicators for environmental impact studies ecological impact studies in Campo Grande, MS

The control of insects, pests on agriculture or vectors for human diseases, is frequently made through the use of insecticides, which are highly toxic. However, nothing is known about the effect of these on non-target insects, such as native bees. The objective of this work is to evaluate the diversity and richness of native bees from Cerrado biome. Malaise traps were installed in a pasture near a fragment of Cerrado, using three repetitions, in raining and hot climate, from October to December 2010. Once a week, for four weeks, samples of bees were taken and conserved in Dietrich solution. The apiforms specimens from each sample were separated in morphospecies and sent to identification. Malaise trap proved to be an excellent method for capturing native bees. The Tribe Meliponini was the most abundant in open areas of pasture and in areas of Cerrado fragment; consequently it is suggested here as a good bioindicator for environmental studies impact. The Meliponini species present were *Reigona* gr. *fulviventris*, *Paratrigona lineata*, *Trigona recursa*, *Partamona* sp, *Trigona spinipes*, *Trigona* gr. *hyalinata*, *Trigona* gr. *fulviventris*, *Tetragonisca fiebrigi*, *Tetragona clavipes*, *Frieseomelitta* cfv *flavicornis*, *Lestrimelitta rufipes*. The dominant species was *Paratrigona lineata*, comprising 82.47% of all species collected.

Table 9 – Numbers of Apidae specimens and other apiforms, number of morphospecies, collected during one week, in Malaise trap, in pasture area around Cerrado fragment, Campo Grande, MS, October, 2010.

Subfamilies	week 1		week 2		week 3		week 4	
	Pasture	Cerrado	Pasture	Cerrado	Pasture	Cerrado	Pasture	Cerrado
Meliponini	11(3)	4(2)	33(5)	10(3)	59(9)	8(3)	20(7)	5(4)
Bombini	1(1)	0	1(1)	0	3(3)	0	0	0
Apini	0	1(1)	0	0	0	0	0	0
Euglossini	0	0	0	0	0	0	1(1)	0
Others apiforms	1(1)	0	2(2)	0	11(4)	0	6(3)	0
Total	13(5)	5(3)	36(8)	10(3)	73(16)	8(3)	27(11)	5(4)
Meliponini (%)	85%	80%	92%	100%	81%	100%	74%	100%

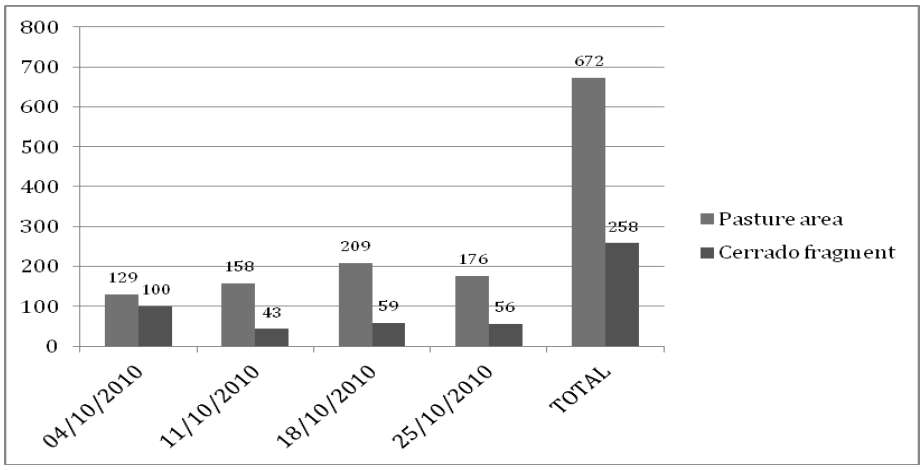

Figure 1 - Populacional flutuation of Meliponini, collected during four weeks in Malaise traps. Campo Grande, MS, october, 2010.

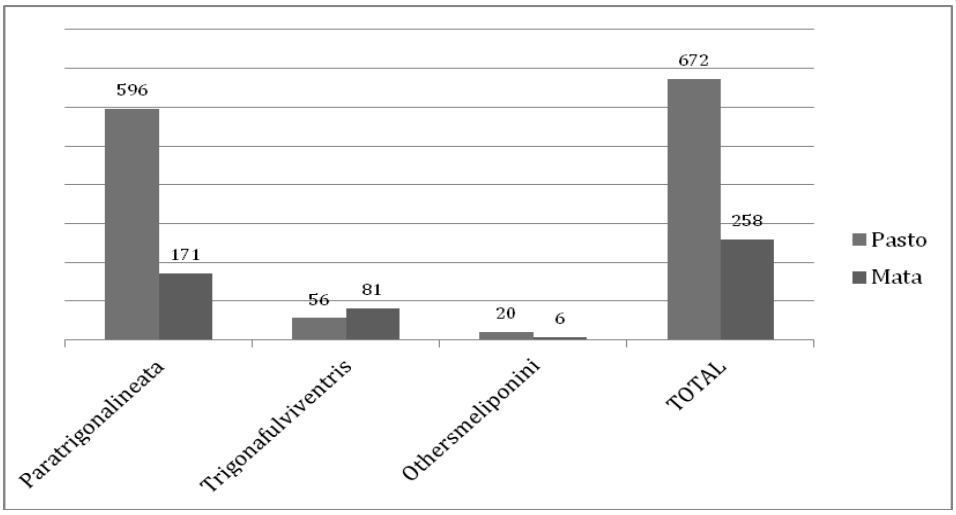

Figure 2 - *Paratrigona lineata*, *Trigona fulviventris* and another Meliponini, in two environmental (Pasture and Cerrado), October, 2010, Campo Grande, MS.

Richness and abundance of Superfamily Hymenoptera parasitoids and predators in different faces in the Pantanal, Corumbá, MS.

3497 specimens of Hymenoptera parasitoids belonging to 10 superfamily were captured. Riparian vegetation in 47 individuals were captured in paratudal 1964 individuals, 383 individuals in Espinheiral and Canjiqueiral 1103 individuals belonging to the times of drought and flood. With these results it was possible to demonstrate the richness and abundance of parasitoids and predators present Hymenoptera these environments.

Table 11. Number of Hymenoptera parasitoids and predators capturados em armadilha de Malaise trap in drought season in diferents environment in Pantanal, Corumbá, MS, mar and april, 2013.

Superfamily	Number of insects			
	Canjiqueiral	Mata ciliar	Paratudal	Espinheiral

Ichneumonoidea	161	4	235	58
Chrysidoidea	33	4	38	18
Evanioidea	5	1	15	6
Platygastroidea	23	0	106	4
Proctotrupoidea	2	0	0	0
Cynipoidea	7	0	55	1
Apoidea	8	2	79	15
Vespoidea	216	0	218	49
Ceraphronoidea	2	0	0	0
Chalcidoidea	215	8	457	44
Total of insects	672	19	1203	195
Number de morfospecies	158	17	177	75

Table 12. Number of Hymenoptera parasitoids and predators capturados em armadilha de Malaise trap in flood season in diferents environment in Pantanal, Corumbá, MS, mar and april, 2013

Superfamily	**Number of insects**			
	Canjiqueiral	**Mata ciliar**	**Paratudal**	**Espinheiral**
Ichneumonoidea	172	7	227	47
Chrysidoidea	3	3	18	5
Evanioidea	4	1	15	1
Platygastroidea	0	1	15	0
Proctotrupoidea	2	0	0	23
Cynipoidea	19	3	82	5
Apoidea	8	4	23	11
Vespoidea	74	4	80	21
Ceraphronoidea	4	0	0	0
Chalcidoidea	145	5	301	75
Total of insects	431	28	761	188
Number de morfospecies	84	19	81	69

CONCLUSIONS

The most abundant Hymenoptera in these two environments were Vespoidea, except for Formicidae, followed by Chrysidoidea and Apoidea Apiformes, mostly in the Cerrado, closely followed by Ichneumonoidea, which are present in both environments. Vespoidea, Apiformes Apoidea and Ichneumonoidea were the most common in all samplings, followed by Chalcidoidea, Chrysidoidea and Apoidea Spheciformes.

It was concluded that the insecticide from Sodium ricinoleate and Malathion are not selective to the Ichneumonidae one week after application. But it two weeks after treatment was not observed there were no differences between treatments and the control.

The dominant species was *Paratrigona lineata*, comprising 82.47% of all species collected. The effect of prototype insecticide – in analisys phase.

The incidence was higher in only two areas and demonstrated that two superfamily were the most abundant, and Chalcidoidea and Ichneumonoidea both in time of drought and flood.

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