CORESTA – STUDY GRANT PARTIAL REPORT

Parasitoid Wasps Applications in Insect Pest Management in Tobacco (*Nicotiana tabacum,* L.) Growing Sites in South Brazil

The entomofauna associated with Organic and Conventional tobacco in Santa Cruz do Sul, with emphasis on parasitoids: the 2008/2009 tobacco harvest

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INTRODUCTION

Integrated Pest Management (IPM) is an effective and environmentally sensitive approach to pest management that relies on a combination of common-sense practices. IPM programs use current, comprehensive information on the life cycles of pests and their interaction with the environment. This information, in combination with available pest control methods, is used to manage pest damage by the most economical means, and with the least possible hazard to people, property, and the environment.

The lack of basic studies (Biology, Taxonomy and Ecology) and data regarding the faunistic composition of insects associated with tobacco crops render and/or retard the progress of research in the area of Integrated Pest Management and biological control in Brazil. Surveys on the Entomofauna's biodiversity, especially the native natural enemies, will support concise decisions in management and conservation programs. Nowadays, there are considerable information on aspects related to the plagues that cause damage in the tobacco culture, but very little is known about the relationships between those harmful insects and their native natural enemies. Thus, once the parasitoids diversity occurring within the tobacco cultivation is determined and showed to be effective in biological control of pests of such culture, the use of parasitoids can be managed and their application can be made on a large scale and low cost to the producer.

The purpose of this project relates to a work that started in the 2007/2008 tobacco harvest in the States of Rio Grande do Sul, Santa Catarina e Paraná, Brazil, and lead to a request for a CORESTA study grant in 2008 in order to continue the research in the 2008/2009 and 2009/2010 tobacco harvests. Therefore, the objectives of the project are: (1) to investigate the community of insects associated with organic and conventional tobacco fields, giving especial attention to natural enemies (parasitoids); (2) to find new data regarding the relationships between host-parasitoids associated to tobacco; (3) to verify the effect of adjacent vegetation to the tobacco field on the populations of insects; and (4) to find species of parasitoids that have potential do be used as biological agents in the control of insect-plagues that occur and damage the culture of tobacco.

In this report, we present the results obtained in the 2008/2009 tobacco harvest and the results that have been reached so far in the 2009/2010 tobacco harvest, as the work continues to be developed.

1 METHODOLOGY

1.1 Tobacco Fields

1.1.1 Organic Tobacco Field: corresponds to an area of approximately 160x85 meters certified as organic, located in the Industrial District of Santa Cruz do Sul, RS, Brazil, and that belongs to JTI/Kannenberg/KBH&C.

1.1.2 Conventional Tobacco Field: corresponds to an area of approximately 70x15 meters that belongs to Profigen do Brasil.

1.2 Disposal of the traps within the tobacco fields:

1.2.1 Organic Tobacco Field: it was determined three sampling lines with three sampling points in each line (point outside, border and inside), as well as a collection point located in the middle of the plantation. In sampling point it was installed a Malaise trap and four Pit-fall traps (Figure 01). The three lines in which the traps were located comprised: a line where the adjacent vegetation is more abundant (about 10-15m), a line with less adjacent vegetation (3-5m) and a line without adjacent vegetation, limited only by another organic tobacco field.

1.2.2 Conventional Tobacco Field: it was determined a single sampling line, with four sampling points (point outside, border, inside and middle). In each collection point were set four Pit-fall traps and a Malaise trap (Figure 02).

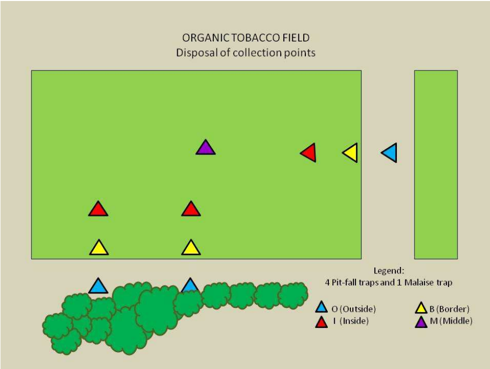

Figure 01: Croquet of the disposal of traps in the organic tobacco field.

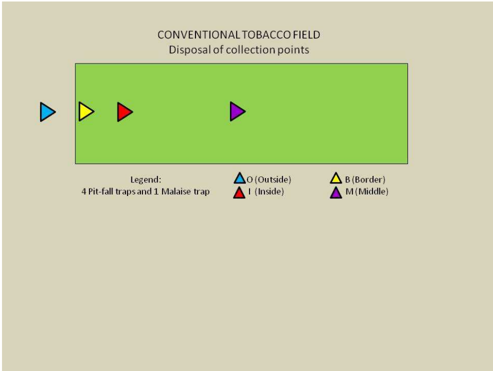

Figure 02: Croquet of the disposal of traps in the conventional tobacco field.

In both tobacco fields, the collections were made on a weekly basis from 20 of November of 2008 to 28 of January of 2009. The material collected was conducted to the Entomology Laboratory of the University of Santa Cruz do Sul for proper identification.

1.3 Storage and Identification of the material

The insects collected were separated according to their order using taxonomic keys, and preserved in alcohol 70% plus 2% formalin in little glass recipients. Each recipient has received two labels: one with details regarding the date and local of collection, taxonomic order, kind of trap in which the insects were collected; and another label containing the lot number so it can be more easily found when needed. The insects belonging to the order Hymenoptera were identified to the level of family, and some to the genus level.

1.4 Collection of parasitized caterpillars and aphids

Weekly collections of tobacco leaves that had caterpillars and aphids attached to them were made. The material was taken to the laboratory, deposited into plastic recipients with a nylon tissue on top while waiting for the emergence of possible parasitoids. When the presence of the parasitoid was noted, those insects were gathered and identified to the level of species.

2 RESULTS AND DISCUSSION

It was sampled a total of 260.936 arthropods, distributed into five major groups: Insecta, Arachnida, Chilopoda, Diplopoda and Crustacea. The Class Insecta represented 98% of total invertebrates sampled. In the organic tobacco field 239.988 arthropods were collected and 20.948 were collected in the conventional field (Table 01).

Table 01 – Total number of invertebrates collected in organic and conventional tobacco field, in Santa Cruz do Sul, RS, Brazil, from 20 November 2008 to 28 January 2009.

Taxon	Organic	Conventional
Hymenoptera	73.920	4.141
Coleoptera	71.849	1.183
Diptera	67.113	12.411
Collembola	7.911	373
Homoptera	5.635	2.963
Lepidoptera	3.777	796
Araneae	1.858	327
Larvas	1.320	35
Acari	805	60
Crustacea	439	25
Ensifera	369	39
Neuroptera	338	6
Thysanoptera	217	215
Opiliones	119	0
Dermaptera	105	8
Caelifera	104	1
Blattodea	67	23
Isoptera	64	4
Heteroptera	47	832
Odonata	20	19
Trichoptera	13	378
Diplopoda	12	1
Psocoptera	10	0
Mantodea	5	1
Pseudoscorpiones	4	0
Scorpiones	0	22
Plecoptera	3	0
Chilopoda	2	1
Ephemeroptera	1	0
TOTAL	**239.988**	**20.948**

The material collected in this research is deposited in the Entomology Collection of the University of Santa Cruz do Sul and comprises the biggest collection of insects and arthropods associated with tobacco in Brazil, serving to academic students as basis for future projects regarding the biological, taxonomic and ecological aspects of the interactions between animal-plant.

2.1 Influence of adjacent vegetation on insect populations associated with tobacco

In this study, the fact that a greater number of invertebrates have been collected at the edge of the tobacco fields, especially on lines one and two of the organic field (Figure 03), show that the adjacent vegetation to the organic farming of tobacco has provided conditions for the insects in the environment, including natural enemies to develop and exercise the natural control of populations of insect pests. The importance of border areas is also visible while observing the pattern of distribution of individuals collected in line three of the organic field, as this line does not have adjacent vegetation, being bordered by another tobacco field.

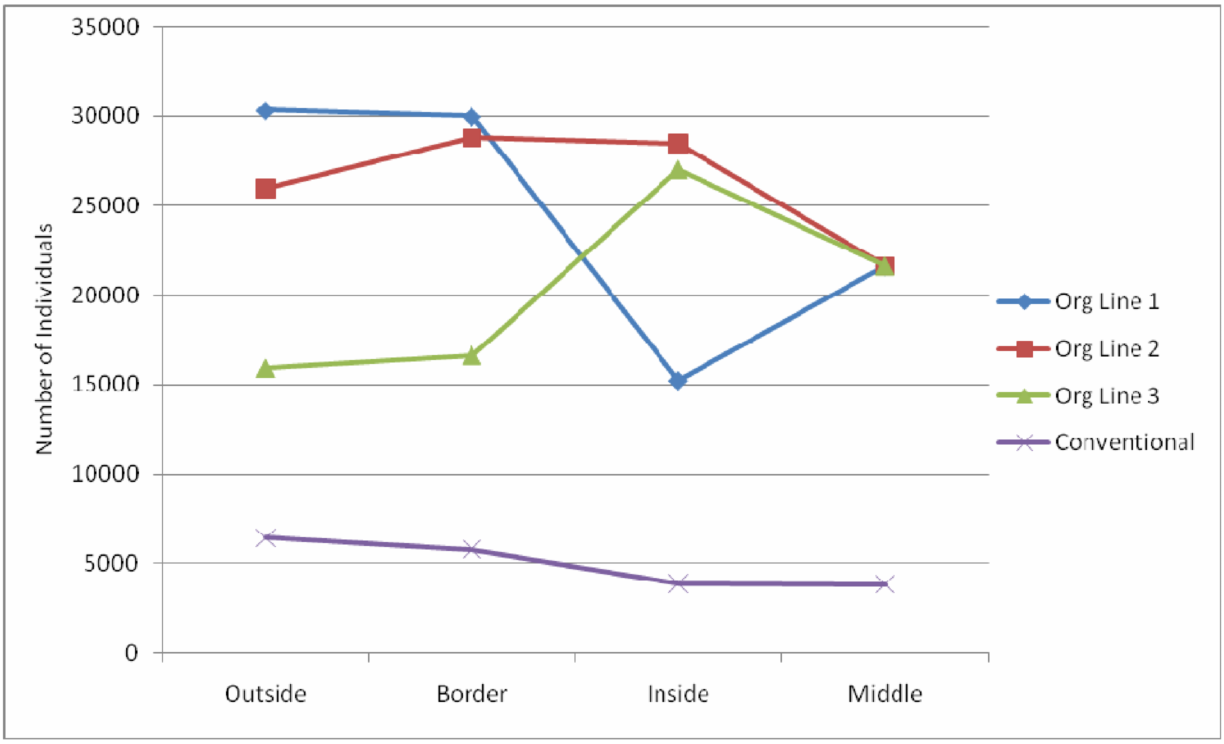

Figure 03 – Graphic of the distribution of invertebrates associated with tobacco, according to the sampling point location.

The increase in the number of individuals collected in the border area supports the idea of surrounding effect on populations of invertebrates, particularly insects, which remain close to these areas near to the main crops due to the fact that they provide food, shelter and nesting sites. Native plant species exert an effect on the populations of insects in the environment, including natural enemies.

The availability of food resources derived from plants such as pollen and nectar can have great impact on populations of insects in crops. Areas of vegetation different from the main crop provide, besides alternative food resources for insects (especially the beneficial ones), moderate microclimate that serves as shelter and protection when environmental conditions become adverse (unfavorable weather - periods of excessive heat or rain seasons; application of pesticides and / or biopesticides) (VENZON *et al.*, 2005). Therefore, these areas of vegetation should be maintained and even encouraged near tobacco fields, as a source of selective resources for natural enemies, without benefit of phytophagous insects.

2.2 Distribution of most common orders according to the location of sampling point and date of collection

2.2.1 Organic Tobacco Field

2.2.1.1 Order Coleoptera

The order Coleoptera is the largest in number of species and currently has more than 300,000 described species, representing about 40% of known taxa of hexapods and 30% of all animal species (COSTA & ROCHA, 2003). In this work, the analysis on the distribution of Coleoptera associated with organic tobacco field according to date of sampling and location of the collection point showed an increase in the number of beetles sampled in the border, inside and middle of the field, by day 13th of january (Figure 04). That is explained mostly because the harvest had started by the area where line three of sampling is located and thus the coleopters moved further inside the tobacco field in order to survive. It is important to note that most species of beetles in agricultural landscapes are concentrated within the crop area since they are adapted to the environment and therefore have the resources they need.

2.2.1.2 Order Homoptera

Specimens of Homoptera that are considered pests in tobacco includes the species *Myzus persicae* (Sulzer, 1776) and *M. nicotianae* Blackman (1987), which hinder the development of the plant by sucking sap and wounded plant tissues, promoting the action of pathogens. The distribution of Homoptera populations according to the date of collection and location of the sampling point showed lower number of homopters collected within the inside and middle areas of the tobacco field (Figure 05). This is due to the fact that, during the tobacco season, there is a higher concentration of nicotine that repels the Homoptera, which prefer environments where they can find alternative food resources from other plant species. Also, it is possible to observe that the life cycles distribution in the outside and border areas has an impact on collection of specimens of Homoptera, while the distribution inside the tobacco field is more uniform.

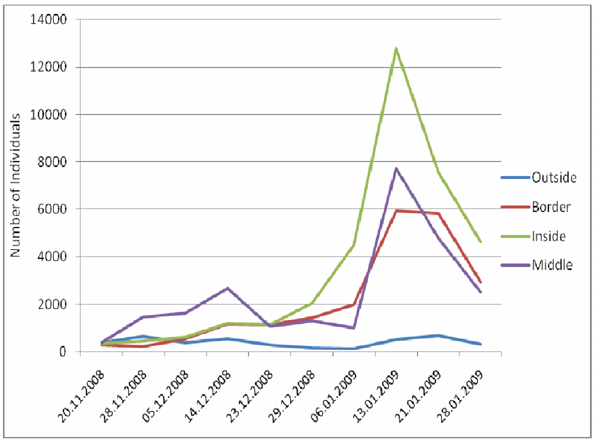

Figure 04 – Graphic of the distribution of Coleoptera in organic tobacco field.

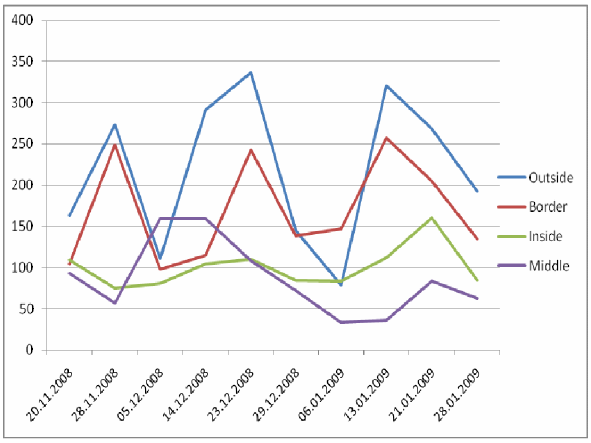

Figure 05 – Graphic of the distribution of Homoptera in organic tobacco field.

2.2.1.3 Order Lepidoptera

The Lepidoptera has great economic importance as the larvae of most species are phytophagous, and many are pests of cultivated plants. In tobacco growing sites, among the larvae of species of Ledidoptera found and sometimes considered pests it is cited the black cutworm [*Agrotis ipsilon* (Hufnagel, 1767)] and the tobacco hornworm [*Manduca sexta* (Linnaeus, 1763)]. It was collected a higher number of specimens of Lepidoptera in the outside and border areas (Figure 06) as the adults of this order prefer environments that differ from the main crop in agroecossistems and, in the case of tobacco, it is more likely to find the larvae of Lepidoptera inside the field as it feeds on the tobacco plant.

2.2.1.4 Order Hymenoptera

The distribution of Hymenoptera specimens along the tobacco crop season and according to the location of sampling point showed a higher number of individuals collected in the middle point and outside point around the beginning of december (Figure 07). The high occurrence of Hymenoptera inside the organic tobacco field is mostly due to populations of ants (Formicidae) which have a foraging habit and are adapted to the environment inside the tobacco field, while the peak of occurrence of Hymenoptera outside the tobacco field in the same time is due to bees and wasps (especially of the families Ichneumonidae and Braconidae) that move in and out of the tobacco field as they can find alternative food resources and nesting sites in those areas.

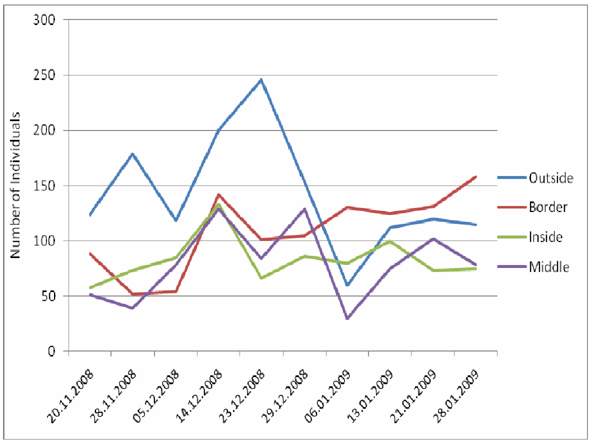

Figure 06 – Graphic of the distribution of Lepidoptera in organic tobacco field.

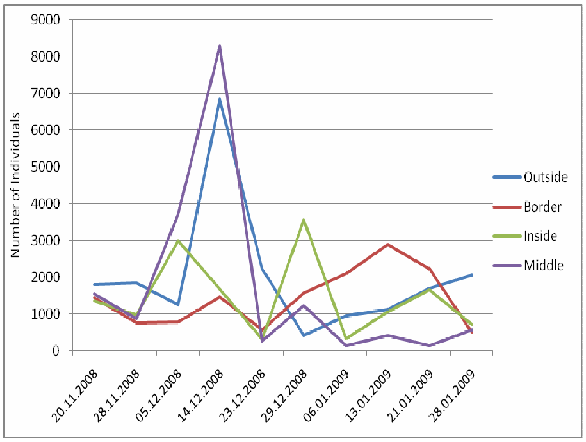

Figure 07 – Graphic of the distribution of Hymenoptera in organic tobacco field.

2.2.2 Conventional Tobacco Field

The pattern of distribution of the orders Coleoptera, Lepidoptera and Hymenoptera in the conventional tobacco field is very similar along the tobacco crop season (Figures 08, 10 and 11). It is clearly observed the impact of the application of pesticide on the number of individuals collected after the second week of december and how the life cycle is reestablished after the beginning of january.

The application of chemical insecticides of broad spectrum is the most widely used technique for the control of pests in Brazil and this method causes a chain mortality of the species present in the area, affecting, directly or indirectly, organisms working in different trophic levels and other ecosystem components, including microbes and soil organisms. This causes a change in interactions between species leading to disruption of food chains and preventing the natural biological control (BARBOSA, 1998). The effects of this phenomenon can be observed in reducing the variety and abundance of main species of predators (mostly of the orders Coleoptera and Hymenoptera) and parasitoids that operate in the system, with consequent increase in population of rare species or that occur sporadically and in the rate of pest resurgence (PIMENTEL *et al*., 1993).

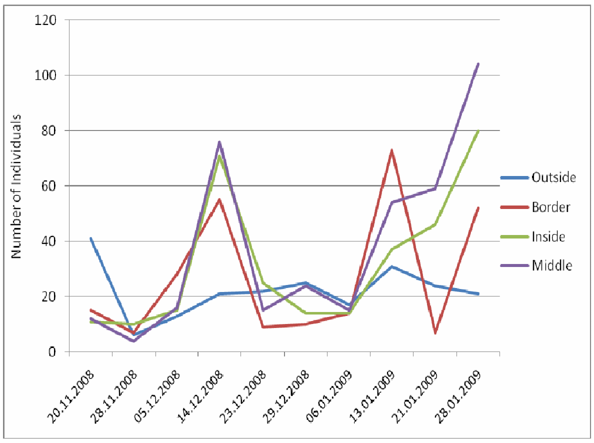

Figure 08 – Graphic of the distribution of Coleoptera in conventional tobacco field.

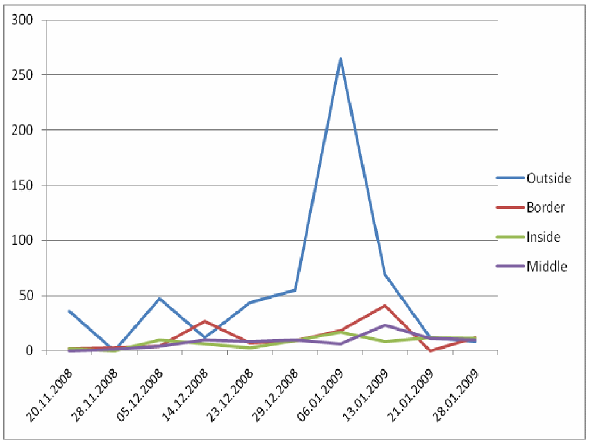

Figure 09 – Graphic of the distribution of Homoptera in conventional tobacco field.

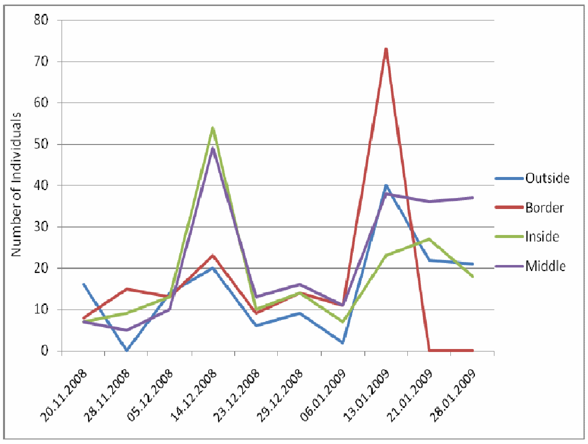

Figure 10 – Graphic of the distribution of Lepidoptera in conventional tobacco field.

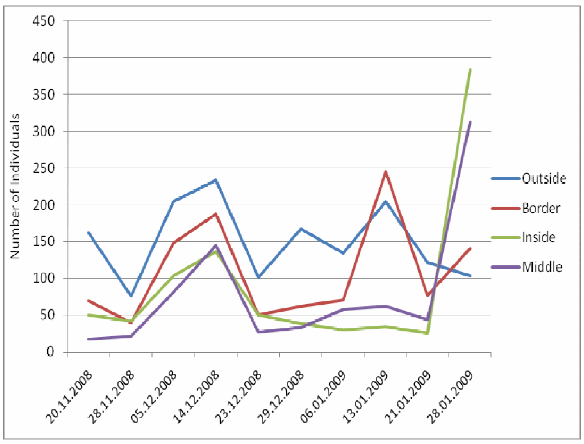

Figure 11 – Graphic of the distribution of Hymenoptera in conventional tobacco field.

2.3 Considerations on Order Hymenoptera

It was sampled 78.081 insects belonging to the order Hymenoptera, of which 55.649 belong to the family Formicidae. The remaining 22.432 individuals are distributed in 47 families (Table 02), and 98.4% of these are part of families considered parasitoids (*).

Table 02 – Families of Hymenoptera associated to tobacco (*Nicotiana tabacum* L.) in Santa Cruz do Sul, RS, Brazil in the 2008/2009 tobacco harvest. (*) Parasitoid families.

Family	Number of Individuals
Agaonidae*	6
Ampulicidae	2
Aphelinidae*	23
Apidae	108
Argidae	7
Bethylidae*	1.145
Braconidae*	5.807
Bradynobaenidae	8
Colletidae	4
Ceraphronidae*	245
Chalcididae*	167
Charipidae*	2
Chrysididae*	22
Crabronidae	56
Diapriidae*	405
Dryinidae*	77
Encyrtidae*	599
Eucharitidae*	4
Eucoilidae*	2.320
Eulophidae*	498
Eupelmidae*	57
Eurytomidae*	160
Evaniidae*	37
Figitidae*	84
Halictidae	86
Ichneumonidae*	5.963
Megachilidae	4
Megaspilidae*	25
Mutillidae*	91
Mymaridae*	568
Nyssonidae	23
Pemphredonidae	5
Pergidae	8
Perilampidae*	39
Platygastridae*	300
Pompilidae*	223
Proctotrupidae*	16
Pteromalidae*	206
Scelionidae*	1.820
Scoliidae	1
Signiphoridae*	19

Sphecidae	16
Tetracampidae*	205
Tiphiidae*	43
Torymidae*	87
Trichogrammatidae*	4
Vespidae	837
TOTAL	**22.432**

In the organic field, 20.145 specimens of Hymenoptera were collected, of which 19.255 belong to 33 parasitoid families. In the conventional field, 2.287 individuals of Hymenoptera were collected and of these, 2.012 are distributed into 28 parasitoid families (Table 03).

Table 03 – Families of hymenopterans parasitoids collected in organic and conventional tobacco fields in Santa Cruz do Sul, RS, Brazil, in the 2008/2009 harvest.

Family	Organic	Conventional
Ichneumonidae	5.573	390
Braconidae	5.382	425
Eucoilidae	2.094	226
Scelionidae	1.591	229
Bethylidae	972	173
Mymaridae	512	56
Encyrtidae	461	138
Eulophidae	432	66
Diapriidae	365	40
Platygastridae	271	29
Pompilidae	202	21
Pteromalidae	189	17
Ceraphronidae	185	60
Tetracampidae	173	32
Chalcididae	151	16
Eurytomidae	147	13
Torymidae	83	4
Figitidae	80	4
Dryinidae	71	6
Mutillidae	65	26
Eupelmidae	51	6
Perilampidae	38	1
Evaniidae	35	2
Megaspilidae	24	1
Chrysididae	22	0
Tiphiidae	22	21
Signiphoridae	19	0
Aphelinidae	16	7
Proctotrupidae	15	1
Agaonidae	4	2
Eucharitidae	4	0
Trichogrammatidae	4	0
Charipidae	2	0
TOTAL	**19.255**	**2.012**

The occurrence of 33 parasitoid families associated with tobacco shows high diversity, considering that there are 61 families of Hymenoptera parasitoids in the world, and that several of these are exclusive of zoogeographical regions such as the Holarctic and Australian, and that 37 families occur in Brazil (AZEVEDO & SANTOS, 2000).

Surveys of Hymenoptera parasitoids are scarce in agroecosystems, but one can affirm the potential on the biodiversity of these insects associated with tobacco crops in Brazil by comparing the present results with those already published on other cultures. Perioto *et al*. (2002a, 2002b) and Souza *et al*. (2006) sampled the occurrence of 15 families associated with the cultivation of soybeans in Nuporanga, São Paulo State and 22 families associated with the cultivation of sorghum, maize, beans and wheat in rotation, respectively. Santos (2008) sampled 28 families of parasitoids associated with the cultivation of coffee in Bahia. This result presents the importance and need of more specific research concerning the biodiversity of native natural enemies within the culture of tobacco and the potential impact that the conservation of these animals within the areas inside and surrounding the main crop field may have.

The families Ichneumonidae, Braconidae, Scelionidae and Bethylidae were the most frequent in both fields, organic and conventional, and individuals of these groups already have been used in biological control programs in Brazil. The families Ichneumonidae and Braconidae are the biggest families of parasitoids in the world and their species are important controllers of populations of Lepidoptera, Coleoptera, Homoptera and Hymenoptera. The species of the genus *Cotesia* Cameron, 1891 are usually found associated with tobacco with its pupae attached to the tobacco hornworm (*Manduca sexta*) and species of the genus *Aphidius* Nees, 1818, parasitize the tobacco aphid.

The egg parasitoids of the family Scelionidae are some of the most important natural enemies of bugs attacking major cultures. The species *Telenomus podisi* Ashmead, 1893 and *Trissolcus basalis* (Wollaston, 1858) have been reared massively in laboratories and been released in soybean fields and there are reports on the interaction of these two species acting together in the control of *Piezodorus guildinii* (Westwood, 1837), *Euschistus heros* (Fabricius, 1798) and *Nezara viridula* (Linnaeus, 1758).

The Bethylids of major importance in agroecossistems in the Neotropical region are two species introduced from Africa to control de Coffee Berry Borer (*Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Scolytidae), which are *Cephalonomia stephanoderis* Betrem, 1960, and *Prorops nasuta* Waterston, 1923 (AZEVEDO, 1999); and there are species that have a special role in controlling populations of ants.

2.3.1 Influence of adjacent vegetation on parasitoids populations

In this study, it was observed that the occurrence of families of hymenopterans parasitoids and the number of individuals caught are higher in the border area of the tobacco field in all lines of sampling, except in line three of the organic field. It should be noted that in the organic field, lines one and two have an area of adjacent vegetation composed of species other than tobacco, and line three has not such edge, being limited only by another tobacco field. Thus, as shown in Figure 12, it is proved the effect that surrounding areas have on the populations of natural enemies, as they can find alternative food resources and place to shelter and nesting near the plant species adjacent to the tobacco crop.

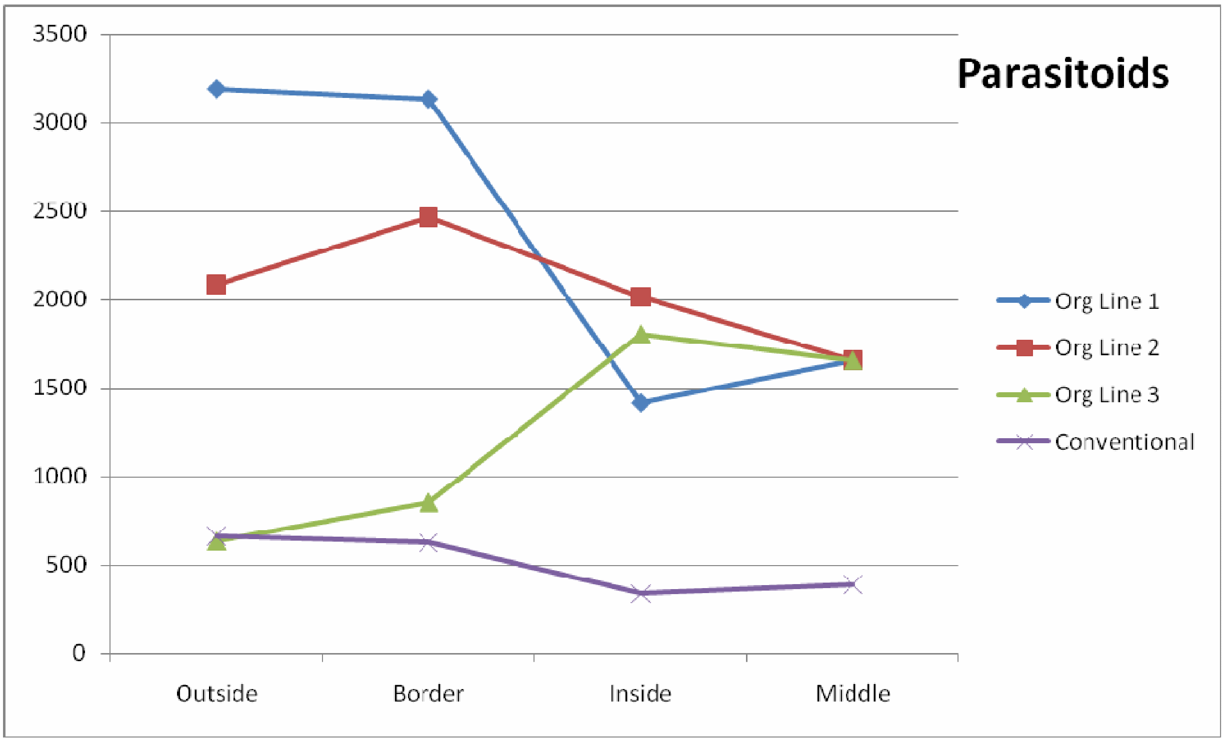

Figure 12 – Graphic of the distribution of parasitoids' populations in organic and conventional tobacco fields, according to the location of sampling point.

In agricultural environments, conservation of natural biological control involves the manipulation of the natural enemies to increase their survival and performance, resulting in increased efficiency (BARBOSA, 1998; GURR & WRATTER, 1999). The diversification of plants in and around the crop area can encourage natural enemies, providing protection from environmental factors and alternative sources of food such as nectar, pollen and honeydew (LANDIS, WRATTEN & GURR, 2000; GURR, WRATTEN & LUNA, 2003).

In this way, the maintenance of areas of embroidery and / or adjacent vegetation to crops provides subsidies to parasitoids occurring naturally in the environment of tobacco. The discovery of the occurrence of such a diversity of native natural enemies associated with tobacco crops in the region of Santa Cruz do Sul, as well as evidence of the impact of surrounding areas on the populations of such individuals is of crucial importance for the implementation of IPM strategies and / or biological control in the culture, with greater chances of success, while seeking to reduce the environmental impacts caused by current farming practices, without causing damage to production.

2.3.2 Parasitoids in Organic X Conventional tobacco field: Statistical analysis

2.3.2.1 Diversity

The Shannon Index of Diversity for the organic field corresponded to 2,149 and for the conventional field it was 2,423, therefore the last one presented higher diversity when it comes to families of hymenopterans parasitoids. This can be explained due to the fact that in the organic tobacco field, although it had a higher number of families occurring, the biological cycles reflect on a less equilibrate distribution of specimens within families, while in the conventional field the individuals' distribution is more uniform. Also, in the conventional field the number of invertebrates collected was lower if compared to the individuals collected in the organic field and that also contributes to a more equilibrate distribution of the specimens within the families.

The hypothesis above cited was tested through the Equitability Index, which measures the pattern of distribution of the individuals within the families, according to KREBS, 1986. For the organic field, the Equitability Index was of 0,6145 which means that the two most abundant families (Ichneumonidae and Braconidae) corresponded to more than 50% of the total number of parasitoids collected. On the other hand, in the conventional field, the Equitability Index was of 0,727 and that assures that the two most abundant families (also Ichneumonidae and Braconidae) corresponded to less than 50% of the total number of parasitoids collected in the conventional tobacco field.

2.3.2.2 Similarity

The analysis of the similarity between the studied areas – organic and conventional tobacco fields was made through the Similarity Index of Sörensen. The index ranges from 0 (no similarity) to 1 (total similarity). The Similarity Index of Sorensen corresponded to 0,92, which proves that the organic field and the conventional field are similar regarding the composition of the families of hymenopterans parasitoids.

2.4 New data regarding host-parasitoid interactions in tobacco

2.4.1 Egg parasitoids associated to tobacco

From 29 of January of 2010 to 28 of February of 2010 leaves with eggs attached to them were collected and taken to the laboratory (Figure 13). It was observed the emergence of eight specimens of *Trissolcus* sp. and 17 *Telenomus* sp. (Hymenoptera: Scelionidae) from eggs, which represent a great possibility on the uses of those species in biological control of Heteroptera eggs attacking tobacco, once those natural enemies are native to the environment and need improvement on their populations. After more research, such increase could be reached through the purchase of this species as they are already created massively in laboratories for future releasing in the tobacco field.

2.4.2 *Trichogramma* sp. (Westwood) and its potential use on biological control of Lepidoptera

The wasps of the genus *Trichogramma* are have been used with success in biological control of *Spodoptera frugiperda* (J.E. Smith, 1797) in maze, soybean and sorghum. The discovery of the natural occurrence of this little wasp associated with tobacco in this project presents the possibility of implementation of biological control of caterpillars that cause damage to the culture through the release of *Trichogramma* in tobacco fields.

2.4.3 Tobacco aphid, *Toxomerus* Macquart, 1855 and *Diplazon* Viereck, 1914

Approximately 50 tobacco leaves with aphids attached to them were collected from 20 of November of 2009 to 29 of December of 2009. Those leaves were taken to the laboratory for the emergence of possible parasitoids. It was noted the parasitism of 34 individuals of *Toxomerus* sp. (Diptera: Syrphidae) and one individual of *Diplazon* sp. (Hymenoptera: Ichneumonidae) on tobacco aphid *Myzus* sp. (Hemiptera: Aphididae). According to published bibliography, the larva of *Toxomerus* predates on *Myzus* and specimens of the genus *Diplazon* parasitize *Toxomerus* thus, presented in this paper the first register of this tritrophic relationship between these genera in tobacco.

2.4.4 Hiperparasitism of *Conura* Spinola, 1837 on *Cotesia congregata* (Say, 1836) in the tobacco hornworm (*Manduca sexta*)

Late in January of 2010 there was a severe occurrence of the tobacco hornworm (*Manduca sexta*) in the organic tobacco field. It was noticed that many caterpillars were parasitized and thus, there were made collections being the material taken to the entomology laboratory for the emergence of parasitoids. From the 12 tobacco hornworm gathered, it was noted the emergence of 616 specimens of *Cotesia congregata* and 19 specimens of *Conura* sp. According to published bibliography, the hiperparasitism of *Conura* sp. in *Cotesia congregata* was already observed in *Spodoptera frugiperda* in maze, and therefore this was the first register of occurrence of hiperparasitism of *Conura* sp. on pupae of *Cotesia congregata* parasitizing *Manduca sexta*.

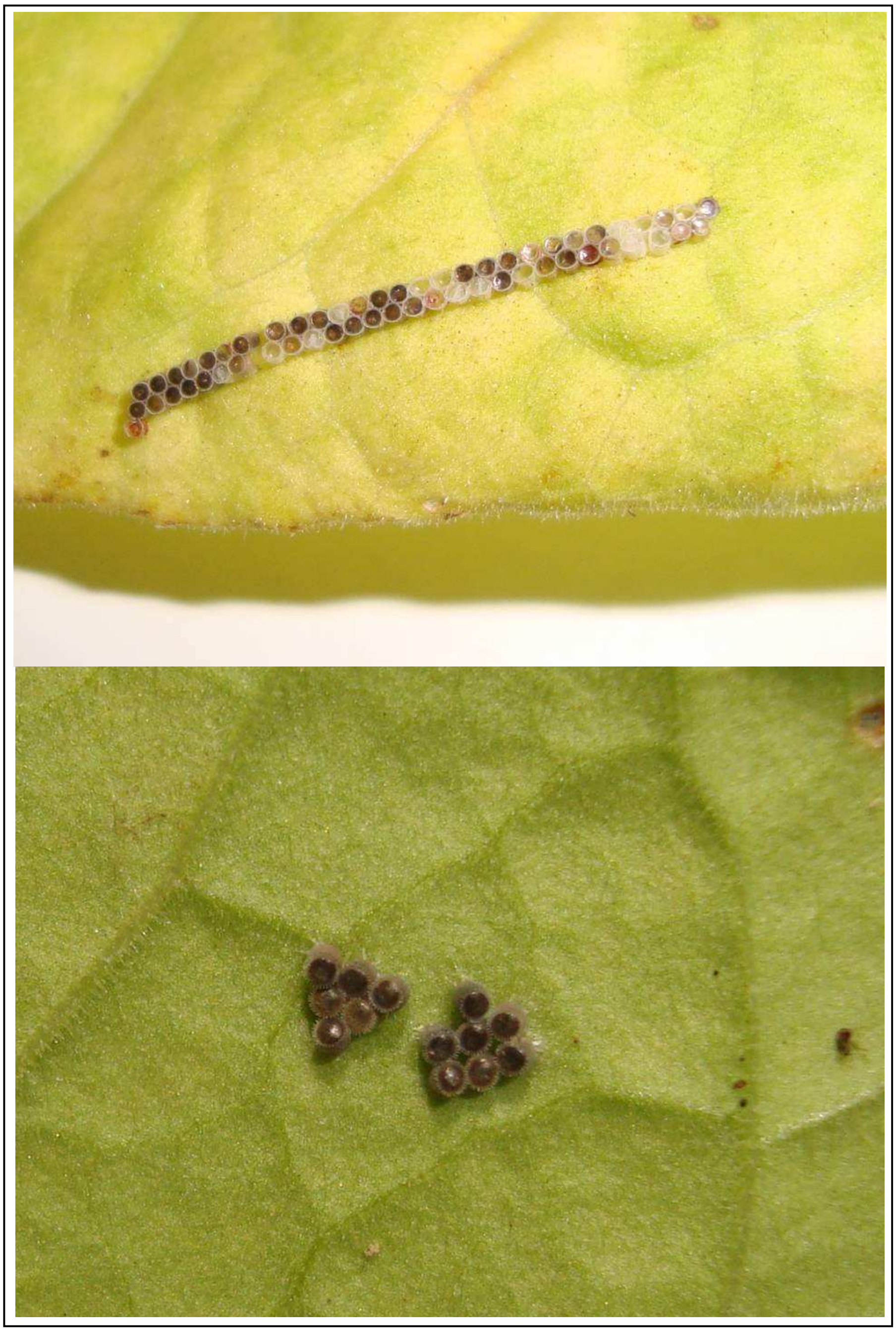

Figure 13 - Parasitized Eggs attached to tobacco leaves.

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